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LIVER AND KIDNEY FUNCTION AFTER PEDIATRIC LIVER TRANSPLANTATION

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ACADEMIC DISSERTATION

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Abstract

Liver transplantation has been an accepted treatment modality for end-stage liver disease in both children and adults for over 30 years. More than 100 children and adolescents have undergone liver transplantation in Finland; one tenth of them have undergone combined liver-kidney transplantation. Life expectancy after pediatric liver transplantation has improved during the years, and six to seven out of ten patients are expected to be alive twenty years later.

Vascular complications, like hepatic artery thrombosis, increase patient morbidity and mortality early after liver transplantation however, the less severe form, late hepatic artery thrombosis, can be asymptomatic. The human leukocyte antigen system is responsible for immune responses against all foreign material, like liver allografts, via various mechanisms. These include production of antibodies, some of which can be directed against the donor, namely donor-specific human leukocyte antigen antibodies. These are harmful to allografts, especially kidneys, but their role in pediatric liver transplantation is less well defined. Long-term complications, including renal impairment, can lead to chronic kidney disease after liver transplantation. Glomerular filtration rate provides an indicator of kidney function more reliably when measured instead of being estimated from surrogate markers.

The aims of this study were to investigate prevalences of late hepatic artery thrombosis with magnetic resonance imaging in 34 patients and donor-specific human leukocyte antigen antibodies with One Lambda LabScreen® antigen immunoassays in 50 patients after pediatric liver transplantation conducted between 1987 and 2007. Long-term renal function was studied with measurement of glomerular filtration rate using plasma clearance of 51-chromium-labeled ethylenediamine tetraacetic acid in 57 pediatric patients who received liver grafts between 1987 to 2007 as well as in 34 pediatric and adult patients who received simultaneously a liver and a kidney between 1993 and 2011.

A decade after pediatric liver transplantation, 44% had late hepatic artery thrombosis and 52% had donor-specific human leukocyte antigen antibodies. Renal function remained stable up to five years and declined thereafter to a mean measured glomerular filtration rate of 66 mL/min/1.73 m² at 15 years after pediatric liver transplantation. In contrast, renal

function was stable during the follow-up, with a mean measured glomerular filtration rate of 70 mL/min/1.73 m² at ten years after pediatric combined liver-kidney transplantation. Instead, renal function was impaired in adult patients after combined liver-kidney transplantation.

In conclusion, late hepatic artery thrombosis and donor-specific human leukocyte antigen antibodies are common after pediatric liver transplantation. Longitudinal renal function remains stable for at least five years after pediatric liver and liver-kidney transplantation, but declines in adult combined liver-kidney transplant patients.

Tiivistelmä

Maksansiirto on ollut myöhäisvaiheen maksanvajaatoiminnan hoitomuoto lapsilla ja aikuisilla yli kolmenkymmenen vuoden ajan. Suomessa yli 100 lasta ja nuorta on saanut maksansiirteen ja kymmenesosa heistä samanaikaisesti myös munuaissiirteen. Lapsena tehdyn maksansiirron jälkeinen eliniän ennuste on parantunut vuosien saatossa ja kuudesta seitsemään lasta kymmenestä on elossa kahdenkymmenen vuoden kuluttua.

Verisuoniperäiset komplikaatiot, kuten maksavaltimotukos, lisäävät potilaiden sairastavuutta ja kuolleisuutta pian maksansiirron jälkeen, mutta kehittyessään myöhemmin maksavaltimotukos voi kuitenkin olla oireeton. Ihmisen immuunijärjestelmä vastaa erilaisten vaikutusmekanismien kautta vastustuskyvystä vieraita aineita, kuten maksasiirteitä, kohtaan. Yksi näistä vaikutusmekanismeista on vasta-aineiden muodostus esimerkiksi elinten luovuttajaa kohtaan. Tällöin näitä vasta-aineita kutsutaan luovuttajaspesifisiksi vasta-aineiksi, jotka ovat haitallisia elinsiirteille, erityisesti munuaissiirteille. Näiden luovuttajaspesifisten vasta-aineiden merkitys lapsena tehdyn maksansiirron jälkeen on kuitenkin epäselvä. Munuaisten toiminnan heikkeneminen on yksi pitkän ajanjakson kuluessa kehittyvä komplikaatio maksansiirron jälkeen, ja se voi johtaa myöhäisvaiheen munuaisten vajaatoimintaan. Yksilön munuaisten toimintaa voidaan arvioida munuaiskerästen suodatusnopeudella, jonka mittaaminen antaa luotettavamman arvion munuaisten toiminnasta sen sijaan, että suodatusnopeutta arvioitaisiin laskentakaavoilla.

Tämän tutkimuksen tarkoituksena oli tutkia myöhäisvaiheen maksanvaltimotukoksen esiintyvyyttä hyödyntämällä magneettikuvausta sekä luovuttajaspesifisten vasta-aineiden esiintyvyyttä lapsena tehdyn maksansiirron jälkeen. Lisäksi pitkän aikavälin munuaisten toimintaa mitattiin hyödyntämällä kromileimatun etyyliidiiniitetraetikkahapon puhdistumaa lapsena maksansiirron ja lapsena tai aikuisena samanaikaisen maksa-munuaissiirron saaneilta potilailta.

Viidellätoista (44 %) potilaalla oli myöhäisvaiheen maksavaltimotukos ja 26:lla (52 %) luovuttajaspesifisiä vasta-aineita lapsena tehdyn maksansiirron jälkeen. Munuaisten toiminta pysyi vakaana viisi vuotta, mutta laski tämän jälkeen keskimääräisen munuaiskerästen suodatusnopeuden ollessa 66 ml/min/1.73 m² viisitoista vuotta lapsena tehdyn maksansiirron

jälkeen. Munuaisten toiminta pysyi vakaana lapsena tehdyn maksa-munuaissiirron jälkeen keskimääräisen munuaiskerästen suodatusnopeuden ollessa 70 ml/min/1.73 m² kymmenen vuoden kuluttua. Munuaisten toiminta sitä vastoin heikkeni maksa-munuaissiirron saaneilla aikuispotilailla.

Johtopäätöksinä todetaan, että maksavaltimotukos ja luovuttajaspesifiset vasta-aineet ovat yleisiä lapsena tehdyn maksansiirron jälkeen. Munuaisten toiminta on tasaista ainakin viisi vuotta lapsena tehdyn maksansiirron tai maksa-munuaissiirron jälkeen, mutta heikkenee maksa-munuaissiirron saaneilla aikuisilla.

Original publications

This thesis is based on four original articles, which are later referred to with their Roman numerals, and reprinted with the permission of copyright owners. Some unpublished data is also presented.

- I Kivelä JM, Kosola S, Kalajoki-Helmiö T, Mäkisalo H, Jalanko H, Holmberg C, Pakarinen MP, Lauronen J. Late hepatic artery thrombosis after pediatric liver transplantation: a cross-sectional study of 34 patients. *Liver Transpl* 2014;20(5):591-600.
- II Kivelä JM, Kosola S, Peräsaari J, Mäkisalo H, Jalanko H, Holmberg C, Pakarinen MP, Lauronen J. Donor-specific antibodies after pediatric liver transplantation: a cross-sectional study of 50 patients. *Transpl Int* 2016;29(4):494-505.
- III Kivelä JM, Räisänen-Sokolowski A, Pakarinen MP, Mäkisalo H, Jalanko H, Holmberg C, Lauronen J. Long-term renal function in children after liver transplantation. *Transplantation* 2011;91(1):115-20.
- IV Kivelä JM, Lempinen M, Holmberg C, Jalanko H, Pakarinen MP, Isoniemi H, Lauronen J. Renal function after combined liver-kidney transplantation: A longitudinal study of pediatric and adult patients. *Pediatr Transplant* 2019 Apr 1:e13400 (Epub ahead of print).

Abbreviations

ARPKD, autosomal recessive polycystic kidney disease

BSA, body surface area

CI, confidence interval

CKD, chronic kidney disease

CLKT, combined liver-kidney transplantation

CNI, calcineurin inhibitors

CsA, cyclosporine A

DSA, donor-specific antibodies

EDTA, ethylenediaminetetraacetic acid

eGFR, estimated glomerular filtration rate

GFR, glomerular filtration rate

HAT, hepatic artery thrombosis

HCV, hepatitis C virus

HLA, human leukocyte antigen

HRS, hepatorenal syndrome

IQR, interquartile range

KT, kidney transplantation

LT, liver transplantation

MELD, model for end-stage liver disease

MFI, mean fluorescence intensity

mGFR, measured glomerular filtration rate

PELD, model for pediatric end-stage liver disease

PH, primary hyperoxaluria

PR, prevalence ratio

RCT, randomized controlled trial

SD, standard deviation

SOT, solid organ transplantation

TAC, tacrolimus

1. Introduction

Organ failure is loss of an organ's ability to maintain normal homeostasis without medical interference (1). Solid organ transplantation (SOT) is a treatment option for organ failure in both adult and pediatric patients. Liver transplantation (LT) is the second most common SOT after kidney transplantation, and the first successful LT was carried out after trial and error in 1967. Combined liver-kidney transplantation (CLKT), in which both the liver and the kidney are procured from the same donor and transplanted one after another, is less common. In the United States, 8,082 LTs and 739 CLKTs were performed in 2017, and of these 599 (7.4%) and 21 (2.8%) were pediatric LTs and CLKTs, respectively (2).

The first pediatric LT in Finland was performed in 1987, and by the end of 2017, 136 children and adolescents had received a liver transplant. Ten percent of these children have undergone CLKT. The most common indication for pediatric LT is biliary atresia, which accounts for about one third of all LTs in pediatric patients in the Nordic countries (3). Over the years, survival rates have improved, with five-year patient survival recently reported to be 81% after pediatric LT based on a single center study (4).

Both short- and long-term challenges can occur after LT. Vascular complications, like hepatic artery thrombosis (HAT), increases patient morbidity and mortality. Pediatric LTs have shown to be complicated by HAT in less than 10% of cases (5). However, a less drastic clinical sequela is possible when HAT occurs late (after 30 days) rather than early after LT, possibly due to collateral vascularization.

Renal impairment is one of the long-term challenges after LT. It is relevant in pediatric patients who are susceptible to the adverse effects of their life-long immunosuppressive medication, especially calcineurin inhibitors (CNIs). Approximately 25% of pediatric patients have been shown to develop renal insufficiency ten years after LT (6). Surveillance of renal function is based on glomerular filtration rate (GFR) by using estimation equations and/or measurement methods, like plasma clearance of 51-chromium-labeled ethylenediaminetetraacetic acid (^{51}Cr -EDTA).

Unlike other solid organs, the liver is considered –at least to some extent– immunologically tolerant to donor-specific human leukocyte antigen antibodies (DSA). However, DSAs have

been shown to be associated with fibrosis in liver biopsy specimens after pediatric LT (7). Roughly, 40% of pediatric patients have DSAs after LT (8) although the clinical relevance of these are somewhat uncertain.

LT is a treatment with large impact. That is, one cannot survive without a functioning liver, and therefore medical interference is necessary to maintain the liver's normal homeostasis. Long-term challenges after LT have become essential not only in the form of patient and graft survival but also in the form of patients' well-being. In this thesis, we aimed to study long-term outcomes after pediatric transplantation focusing on the prevalence of late HAT (study I) and DSAs (study II) after pediatric LT and renal function after pediatric LT (study III) and CLKT (study IV).

2. Review of the literature

2.1 Liver and kidney

2.1.1 Anatomy and function

The liver is the largest internal organ, located principally on the upper right side within the abdominal cavity. It weighs around 1.4 to 1.6 kilograms in adults and around 350 to 400 grams in one-year-old infants (9).

It is anatomically divided into a right and a left lobe (Figure 1), and further, into eight functional segments I-VIII. Around 80% of blood inflow to the liver is via the portal vein and the rest via the hepatic artery. Bile outflow from the liver is via a common hepatic duct, which merges with the cystic duct to form a common bile duct.

The liver has numerous tasks, which are chiefly completed by hepatocytes, which are the main cell type in the liver parenchyma. These tasks include synthesis and secretion of bile, maintenance of glucose homeostasis, and synthesis of numerous proteins, such as albumin, lipoproteins and coagulation factors, among others (10).

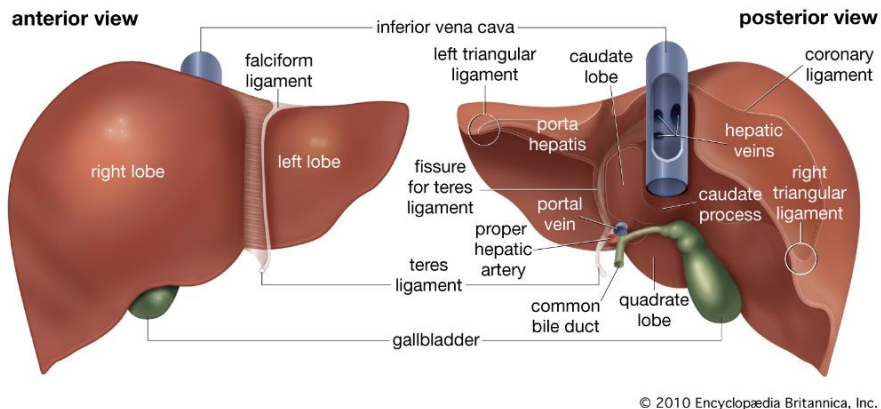


Figure 1. The liver seen anteriorly (left) and posteriorly (right). By courtesy of Encyclopædia Britannica, Inc., copyright 2010; used with permission.

The bean-shaped kidneys are located on both sides of vertebra within the upper part of the abdominal cavity in the retroperitoneal space. They weigh around 120 to 170 grams in adults and 60 to 80 grams in one-year-old infants (9).

The structure of a kidney is shown in Figure 2. It has been estimated that each kidney of a healthy adult has, on average, 860,000 nephrons (11), which are key functional units of the kidney (Figure 2B). Each nephron is composed of a glomerulus (number 3 in 2B) and a tubule (numbers 4 to 8 in 2B). Roughly, 180 liters of ultrafiltrate is formed in glomeruli each day, and almost all of it is reabsorbed in tubules in such a way that final the urine output is normally about 1 to 2 liters per day.

The kidney has various functions, such as control of water retention, sodium excretion, vitamin D, renin and erythropoietin synthesis (12).

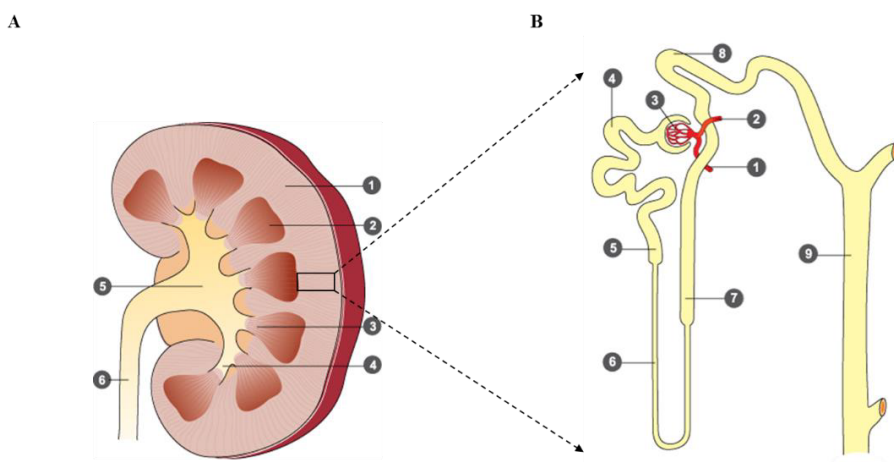


Figure 2. Cross-section of kidney (A) and magnified presentation of nephron (B). The numbers in (A) denote 1) cortex 2) medulla 3) papilla 4) minor calyx 5) renal pelvis and 6) ureter. The numbers in (B) denote 1) afferent arteriole 2) efferent arteriole 3) glomerulus 4) proximal convoluted tubule 5) proximal straight tubule 6) descending (thin) limb of loop of Henle 7) thick ascending limb of loop of Henle 8) distal convoluted tubule and 9) collecting duct.

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2.1.2 Measuring liver and kidney function

2.1.2.1 Liver

Various approaches exist to evaluate a suspected liver disease, including the use of serum- or plasma-based laboratory tests. In clinical practice, commonly used laboratory tests can broadly be categorized into those that reflect hepatocellular injury (for example alanine aminotransferase; ALT), cholestasis (for example bilirubin), and synthetic function (for example prothrombin time) (13). An assorted number of liver diseases, like acute viral hepatitis and primary biliary cirrhosis, can result in abnormal laboratory test results (13). Nonetheless, these laboratory tests are not specific to certain liver diseases, which is why using more than one diagnostic approach is warranted, including liver biopsies and radiological imaging.

2.1.2.2 Kidney

Evaluation of renal function is based on the glomerular filtration rate (GFR), which is the rate at which ultrafiltrate is formed from blood when passing all glomerular capillaries (14). GFR is reported as milliliters per minute per standardized body surface area (BSA) ($\text{mL/min}/1.73 \text{ m}^2$) and is, on average, around $125 \text{ mL/min}/1.73 \text{ m}^2$ in healthy young Caucasian adults (14). It should be noted that GFR normally declines with age after the first two years.

Measuring the glomerular filtration rate (GFR) directly is impossible. Nonetheless, GFR can be measured indirectly with renal or plasma clearance of exogenous filtration markers, like ^{51}Cr -labeled ethylenediaminetetraacetic acid (^{51}Cr -EDTA). GFR is equivalent to clearance when no reabsorption or secretion of filtration markers occurs in tubules. There are several different exogenous filtration markers, some of which are more reliable than others compared to the standard of renal inulin clearance (15). Detailed methods to measure GFR (mGFR) with the use of plasma clearance of ^{51}Cr -EDTA have been reported elsewhere (16). In Finland, GFR has been measured with plasma clearance of ^{51}Cr -EDTA in pediatric SOT patients.

In addition to indirect measurement, GFR can be estimated (eGFR) with the use of estimation equations that are based on plasma or serum levels of an endogenous filtration marker, such as commonly used creatinine. Diet, age and muscle mass (i.e. so-called non-GFR determinants) influence plasma or serum creatinine levels (14).

There are numerous GFR estimation equations (17, 18), such as the serum creatinine-based Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation developed for adult patients (19) and the bedside Schwartz equation developed for pediatric patients (20).

In clinical practice, the rationale for using eGFR is its effortlessness compared to mGFR, which is ponderous, especially in pediatric patients. Still, mGFR provides the best estimate of true GFR. Evaluation of GFR is usually complemented with urinalysis, radiological imaging and kidney biopsies if renal disease is suspected.

2.2 Pediatric liver transplantation

2.2.1 The early days

The history of LT along with the timeline of achievements has been published elsewhere (21). The first attempted LT in a human was performed on a three-year-old patient in 1963, but the patient died during the operation (21). Between 1963 and 1964, six other LTs were attempted in adults, but all died either during the operation or within a few weeks following it (21). The first considered successful LT was also performed on a pediatric patient in 1967, and the patient survived beyond one year (22). The immunosuppressive drug cyclosporine was introduced for clinical use in 1979 and LT was accepted as a treatment option in 1983 at a consensus conference held in the United States (21). In Europe, number of LTs increased after the early 80s (23). In Finland, the first pediatric LT was performed in 1987, a few years after the first adult LT in 1982.

2.2.2 General aspects of organ donation and allocation

In Finland, most solid organs are procured from brain-dead donors and a small proportion (around 13%) of kidneys from living related donors. On average, four organs per brain-dead donor are procured in Finland (24). Contraindications for donation after brain death are

unknown cause of brain death, age over 85 years, viral hepatitis B or C, HIV, and malignant tumor diagnosed in the past five years (excluding basal cell carcinoma or brain tumor).

In some European countries and United States, 12% to 15% of deceased donors are non-heart beating donors (25, 26). A part of the liver can also be resected from a living person (i.e. living donor LT; LDLT) although this mode of LT is not used in Finland. In Europe and United States, 4% to 11% are LDLTs (23, 27).

In contrast to other forms of SOT, ABO blood group but not human leukogen antigen compatibility between donor and recipient is a prerequisite for LT if the donor is otherwise suitable. Additionally, body size compatibility is needed in the case of pediatric LT with an optimal weight of the liver graft around 1% to 3% of the recipient's weight (28). However, half of the deceased pediatric liver donors who are initially declined because they are considered to be size mismatched are within range as per donor and recipient body surface area ratio (29).

Organ and liver allocation policies in Scandinavia, Europe and United States have been detailed elsewhere (3, 25, 29). Briefly, liver allocation in the United States is based on medical urgency categories (i.e. status 1A or 1B or non-status 1A and 1B patients), donor age and geographical area (i.e. local, regional or national) (29). In addition, within non-status 1A and 1B patients, allocation is prioritized based on scores calculated with the Model for End-Stage Liver Disease (MELD) or the Pediatric End-Stage Liver Disease (PELD) equations (29). These equations predict a transplant candidate's probability of dying within the next 90 days.

Obligatory liver exchange and payback rules exist considering, for example, acute liver failure and pediatric patients between Scandiatransplant member countries, including Finland (30). Contrary to the United States and some European countries, MELD and PELD scores are not used for liver allocation in Scandinavia. It should be noted that only one transplant center exists in Finland, providing opportunity to choose donors as well liver recipients locally.

2.2.3 Liver transplantation candidate evaluation

Evaluation of pediatric patients for LT by a multidisciplinary team includes verification of diagnosis, assessment of primary liver disease and other potential organ complications, identification of both absolute and relative contraindications for LT, as well as an appraisal of nutrition status and psychosocial aspects (31).

2.2.4 Surgical procedure

Details of pediatric LT have been described elsewhere (32). Shortly, the recipient operation involves a pre-anhepatic phase in which a native liver is dissected and mobilized, anhepatic phase in which the native liver is removed and a donor liver orthotopically implanted, and a post-anhepatic phase in which blood inflow to the implanted liver is recovered.

Whole liver grafts are transplanted either using piggyback (Figure 3a) or caval replacement (Figure 3b) techniques. In the piggyback approach, the recipient's inferior vena cava remains intact in contrast to caval replacement. Across Europe, 50% and 40% of transplant centers utilize piggyback and caval replacement as a first-line approach, respectively, and the rest on case-by-case basis (33). Only few, small randomized controlled trials (RCT) have tested the superiority of these techniques over each other with inconclusive results (34). During the post-anhepatic phase, the common bile duct is anastomosed in an end-to-side fashion to the jejunum (Roux-En-Y hepaticojejunostomy; Figure 3) or in an end-to-end fashion to the common bile duct.

In addition to whole liver grafts, technical variant liver grafts include split grafts, reduced-sized grafts and living donor grafts. The split liver graft is typically divided between adult and child so that the child receives a smaller portion of the liver (i.e. segments II and III) (32). The main benefit of this approach is to enlarge the donor pool since one graft is shared between two recipients. In the United States, 22% and 14% of pediatric liver recipients receive reduced-sized and split liver grafts, respectively (27). The use of split and reduced-sized liver grafts leads to somewhat inferior patient survival compared to the use of whole liver grafts (35, 36).

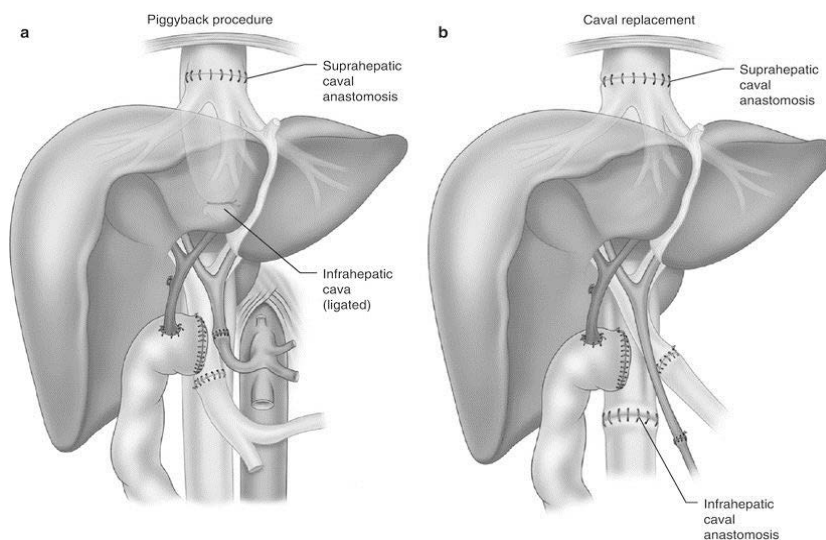


Figure 3. Piggyback approach (left) in which donor's suprahepatic vena cava is sewn to recipient's hepatic veins and donor's infrahepatic vena cava is closed. Conventional (i.e. caval replacement) approach (right) in which donor's suprahepatic and infrahepatic vena cava is sewn to recipient's suprahepatic and infrahepatic vena cava, respectively.

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2.2.5 Indications

The most common primary indication for pediatric LT is biliary atresia (BA), which is a rare congenital cholangiopathy. In Finland, roughly one newborn for every 20,000 livebirths is affected by BA (37). First-line treatment of BA is portoenterostomy although a large proportion of BA patients still need LT after portoenterostomy (38).

Biliary atresia accounts for 30% to 60% of pediatric LTs (3, 23, 26), but only 3% to 4% of all LTs (3, 23). Other main indications for pediatric LT are metabolic diseases, such as tyrosinemia and Wilson's disease, acute liver failure typically due to unknown cause, and liver tumors, such as hepatoblastoma. Patient survival rates vary depending on the primary indication for pediatric LT in such a way that patients with BA or metabolic disease have a

higher probability of surviving compared to patients with acute hepatic failure or hepatoblastoma (27).

2.2.6 Long-term outcomes after liver transplantation

2.2.6.1 General

Liver transplantation poses both short and long-term challenges to these patients. A detailed description of short-term complications is outside the scope of this thesis. These include surgical complications, such as vascular and biliary-related complications, and non-surgical complications, such as primary non-function of the liver graft or acute rejection as well as infections. Table 1 shows the main causes of re-transplantation for a mixture of adult and pediatric patients up to one month after their first LT in Europe.

Table 1. Causes of re-transplantation during the first month after first LT

<u>Indication for re-transplantation</u>	<u>Re-transplantations, n (of total %)</u>
Primary non-function	1,413 (50.4)
Surgical	1,004 (35.8)
Vascular complications	924 (33.0)
Rejection	185 (6.6)
Acute rejection	170 (6.1)
Sepsis	62 (2.2)
Bacterial infection	47 (1.7)

Note. Sum of percentages not 100% due to omission of other causes. Modified from ref 23.

Primary non-function and vascular complications account for most of the indications for re-transplantations during the first month after LT in both adults and children (23, 39). Other complications in pediatric patients include biliary, pulmonary and renal complications within the first month after LT (40). Approximately, one third of all pediatric patients

have at least one acute rejection during the first year after LT (27). Most of these rejection episodes are medically manageable.

The most common reason for death after pediatric LT during the first year is cardio- and cerebrovascular related (27). Five-year patient and graft survivals vary from 81% to 86% and 67% to 80% after pediatric LT, respectively (4, 23, 27). A vast number of different risk factors for death and graft failure in pediatric LT patients have been postulated, including transplantation era, organ type, renal replacement therapy, primary diagnosis and growth impairment (4, 36, 41).

Long-term complications after pediatric LT are multifactorial although commonly related to immunosuppressive medication. Long-term complications include post-transplant lymphoproliferative disorder (PTLD), renal impairment, diabetes and growth failure. Of 167 pediatric LT patients who survived 10 years, 53 (32%) had stable liver allograft function, no immunosuppression-related comorbidities (including PTLD, diabetes, renal impairment and growth failure) and no additional medications (42). According to a systematic review, self-reported health-related quality of life is inferior in pediatric LT patients compared to healthy counterparts but comparable to other SOT patients (43).

2.2.6.2 Hepatic artery thrombosis (HAT)

An occlusion within the hepatic artery compromises blood inflow to the allograft, which potentially leads to death without treatment. The diagnosis of HAT is based on radiological imaging modalities, including ultrasonography (US) with specific diagnostic criteria highlighted (44), although US becomes less sensitive after some time to detecting HAT (45).

HAT is the most common form of vascular complication after LT, affecting 3% to 8% of LT patients (5, 46-49). HAT is also more common in children compared to adults, affecting 8% of pediatric patients after LT (5, 46) but affected as much as 42% of pediatric LTs in the past (50).

Hepatic artery thrombosis is divided into an early and late form based on the time elapsed since LT. Despite the fact that this time period varies arbitrarily between studies, most studies have adopted a period of one month (46, 51). Some studies have reported a higher

incidence of early than of late HAT, but the opposite is true for some other studies (5, 47, 52, 53). Late HAT complicates around 1% to 4% of LTs in studies (5, 47, 52, 54-59) with more than 500 LTs (Table 2).

On average, 16 late HATs for every 1,000 LTs were observed after one-month in these nine studies. However, time from LT to diagnosis of late HAT varies even within studies from a few months to years (55, 58). Almost 900 pediatric LTs were included in the largest study shown in Table 2, but unfortunately, late HAT cases were not reported separately for pediatric patients (5). Late HAT incidence (> 1 month) in pediatric patients ranges from 3% to 9% based on a few old studies (50, 60).

Table 2. Incidence of late HAT after 1 month in studies with over 500 LTs

Study (ref) ^a	Patients	Late HAT / LTs (%)
Duffy (5)	adults and children	70 / 4,234 (1.7)
Pareja (47)	adults	16 / 1,674 (1.0)
Stange (52)	adults ^b	16 / 1,192 (1.3)
Silva (54)	adults ^b	39 / 1,257 (3.1)
Gunsar (55)	not specified	11 / 704 (1.6)
Leithead (56)	not specified	80 / 2,047 (3.9)
Scarinci (57)	adults	6 / 739 (0.8)
Vivarelli (58)	adults	13 / 747 (1.7)
Yang (59)	adults	6 / 744 (0.8)
Total		257 / 13,338
Incidence per 1,000 LTs		16.3 (95% CI 11.0 to 24.0) ^c

a studies extracted from ref 51. Bhattacharjya et al (53) excluded due to overlapping patients with Silva et al (54).

b some patients were < 18 years based on reported age range

c average incidence calculated with R 3.1.1 using meta package

There are manifold risk factors reported to be associated with early HAT including donor and recipient related, perioperative care related, and surgical technique related factors (46). For example, low donor-to-recipient age ratio, longer operation time, use of whole grafts, use of reduced-sized grafts, and multiple hepatic artery reconstructions have been

reported to be associated with early HAT in pediatric patients (60-63). In contrast, cytomegalovirus infection, a combination of male recipient and female donor, cause of donor death, re-transplantation and rejections have been suggested to be associated with late HAT (58, 59, 64). Few studies have focused solely on pediatric patients with late HAT.

Early and late HAT can have different clinical course with a less severe picture emerging from late HAT, which can be asymptomatic (47, 52, 53), although elevated transaminases and biliary complications are frequent (5, 47, 52, 53). In a recent study, 50% of patients with late HAT (defined as occurring after three months) were asymptomatic, 18% had mild and 32% had severe symptoms highlighting different sequelae even within these patients (51).

A sufficient formation of compensatory collaterals after LT might explain the different clinical consequences of HAT. Collaterals can be evident as early as 10 hours after ligation of the hepatic artery (65) but also within a few weeks after pediatric LT (50). Fifty-four percent of adult LT patients with HAT demonstrate arterial flow via collaterals (66). Collaterals can introduce false negative findings which mitigate the sensitivity of US (45, 67). The underlying reasons for collateral formation remain elusive although site of thrombosis, time elapsed after LT and type of graft have been suggested (68). In contrast, factors associated with spontaneous neovascularization of the liver graft include late HAT (69).

The use of antiplatelet prophylaxis with aspirin has been reported to be associated with lower incidence of late HAT in one study (70) but not in others (71). Vivarelli et al showed that 0.4% of patients with aspirin had late HAT compared 2.2% without aspirin (70). Contrary, Shay et al demonstrated that 1.2% of patients with aspirin had late HAT compared to 1.0% without aspirin (71). Pooling these two studies yields inconclusive result with a risk ratio of 0.5 (95% CI 0.1 to 3.2) for late HAT with aspirin compared to without. Pediatric studies have not supported an association between aspirin usage and lower HAT incidence (72).

Antiplatelet prophylaxis is typically used for 3 months after pediatric LT although various approaches prevail (73). There are no RCTs that have tested the efficacy of antiplatelet prophylaxis in preventing HAT after LT.

2.2.6.3 Renal function

Chronic kidney disease (CKD) is a well-characterized complication in both adult and pediatric patients after LT as well as after other forms of SOT. Impaired renal function might ultimately progress to end-stage kidney disease (ESKD) requiring dialysis or kidney transplantation (KT). Long-term renal impairment can be caused by a myriad of factors including calcineurin inhibitor (CNI) nephrotoxicity, hepatorenal syndrome (HRS) before LT, hypertension and older age (74).

CKD is defined by the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines as abnormalities of kidney structure or function, present for 3 months, with implications for health (75). The assessment of CKD is based on level of albuminuria, cause and GFR, which is classified into six categories (75) (Table 3). In the previous guidelines (76), there were five GFR stages of which stage three (i.e. GFR 30 to 59) was subdivided into two categories (i.e. G3a and G3b).

Table 3. GFR classification according KDIGO 2012 guidelines

GFR (mL/min/1.73 m ²)	GFR category	Explanation
Equal or more than 90	G1	Normal or high
60 to 89	G2	Mildly decreased
45 to 59	G3a	Mildly to moderately decreased
30 to 44	G3b	Moderately to severely decreased
15 to 29	G4	Severely decreased
Less than 15	G5	Kidney failure

Modified from ref 75.

Sixteen percent of adult non-renal SOT patients develop chronic renal failure, which increases hazard of death around five-fold compared to patients without chronic renal failure (77). With varying definitions and methods, the five-year cumulative incidence of chronic renal failure is 10% to 22% after adult LT (77-79). In Finland, the incidence of ESKD (eGFR less than 15 or dialysis/KT) is 1.8% in adults five years after LT (79).

Three percent of pediatric non-renal SOT patients develop ESKD, which increases hazard of death around three-fold compared to pediatric SOT patients without ESKD (80). The cumulative incidence of ESKD after pediatric SOT is shown in Figure 4 and is around to 1% for pediatric LT patients up to ten years after transplantation based on a large registry study with 8,958 pediatric LT patients (80).

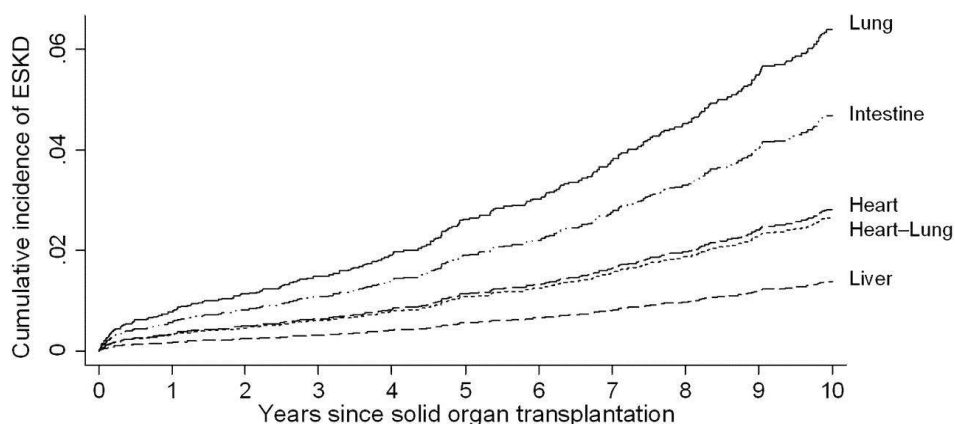


Figure 4. Cumulative incidence of end-stage kidney disease (ESKD) (dialysis or kidney transplantation) after pediatric solid organ (SOT) transplantation stratified by organ. Reproduced with permission from *Pediatrics*, Vol.132, Pages 1319-26, Copyright © 2013 by the AAP.

Risk factors for ESKD in pediatric LT patients include eGFR less than 60 mL/min/1.73m² before transplantation and older age at the time of LT, but not type of immunosuppressive therapy (81).

Although ESKD is infrequent in pediatric LT patients, less severe renal impairment is more common. According to one study, 28% of pediatric LT patients have renal dysfunction one year after transplantation (82). Chronic renal insufficiency (defined as GFR < 60 mL/min/1.73 m²) affects 25% of pediatric LT patients up to ten years after transplantation based on mGFR (6). On the contrary, 18% of pediatric patients have mGFR less than 90 mL/min/1.73 m² five years after LT (83). In this cross-sectional study by Campbell et al,

below normal height at one-year, older age at LT and the use of cyclosporine at one year was associated with increased odds for having mGFR less than 90 mL/min/1.73 m² (83). Since GFR reflects a continuum of renal function, it is straightforward to analyze it in a continuous manner. Single-center studies (6, 84, 85) with mGFR and longitudinal follow-up up to ten years after pediatric LT are shown in Table 4.

The initial number of patients in these studies varied from 37 to 101 and median follow-up from 6 to 9 years. Absolute mGFRs without BSA correction were reported to improve (foot-note in Table 4) over the years in one of these studies (85), which underscores maturation of kidneys in smaller children.

Table 4. Single-center studies with mGFR and follow-up up to ten years after pediatric LT

Study (ref)	GFR method	GFR (mL/min/1.73 m ²)		
		Year 1	Year 5	Year 10
Harambat (6)	Inulin	100	87	71 ^a
Herzog (84)	^{99m} Tc-DTPA	110/85 ^b	105/60 ^b	100/90 ^b
Herlenius (85)	⁵¹ Cr-EDTA	88 ^c	86 ^c	93 ^c

a 10 and 11 years pooled in study
b approximated from figure for others/tyrosinemia patients.
c absolute GFR (mL/min) 42, 56 and 79 at 1, 5 and 10 years, respectively.

Other pediatric studies with mGFR have reported either stable or declining renal function after LT with a follow-up of 3 to 6 years (86-89). Adult LT studies based on mGFR have reported relatively stable GFR up to ten years after transplantation after an initial post-operative fall (90, 91).

The use of GFR estimation equations overestimates or underestimates measured GFR after pediatric and adult SOT including LT (17, 18, 92, 93). However, some equations perform better than others. Ultimately, overestimation as well as underestimation leads to reclassification of patients into different GFR stages or categories (93, 94). On average, eGFR is

around 7 to 40 mL/min/1.73 m² higher than mGFR in pediatric liver and heart transplantation patients depending on the equation used (89, 93, 95). However, for some individual patients overestimation can be considerable (89).

The original Schwartz equation published in 1976 was revised in 2009 with introduction of one constant for all children to be used for GFR estimation (20). In addition, an equation based on height, gender, serum creatinine, cystatin C and blood urea nitrogen was developed (i.e. CKiD equation III) (20). However, the revised (i.e. bedside) Schwartz equation falls behind compared to mGFR and CKiD equation III in pediatric SOT patients, including LT patients (93, 96).

Estimated GFR based on bedside Schwartz equation is deemed acceptable for regular renal function assessment after pediatric LT with focus on a GFR threshold of 70 mL/min/1.73 m² for renal function sparing strategies (97). In contrast, mGFR is regularly used for monitoring renal function in all pediatric SOT patients in Finland.

2.3 Combined liver-kidney transplantation (CLKT)

Combined liver-kidney transplantation is a treatment option for patients with end-stage organ diseases affecting both the liver and the kidney. The first CLKT was conducted in late 1983 in an adult patient with concomitant chronic rejection of a kidney graft and hepatitis B infection (98). One decade later, the first CLKT was a reality in Finland. In 2017, 10 (0.5%) of a total 2,004 patients were transplanted with combined liver and kidney in Scandinavia (99).

The decision which patients with renal dysfunction benefit most from CLKT or isolated LT is not straightforward and depends on either a hepatocentric or nephrocentric approach (100). In the United States, a new allocation policy was implemented in 2002 with the inclusion of the MELD equation (101). This led to an increase in CLKTs in adults since serum creatinine is one of the variables included in MELD equation; higher probability of death is therefore calculated for those patients with poorer renal function (100, 102). Since 2002, different criteria have been proposed for selecting patients for CLKT, the newest one in 2017 including criteria for CKD with GFR 60 or less for at least 3 months with recent GFR

less than 30 or dialysis, or for acute kidney injury with GFR 25 or less over 6 weeks detailed once a week (103). However, the decision often has to be made on an individual basis. In CLKT, both the liver and the kidney are procured from the same donor so that the liver is transplanted first as in isolated LT depending on donor and recipient size match, after which the kidney is transplanted as in isolated KT (Figure 5).

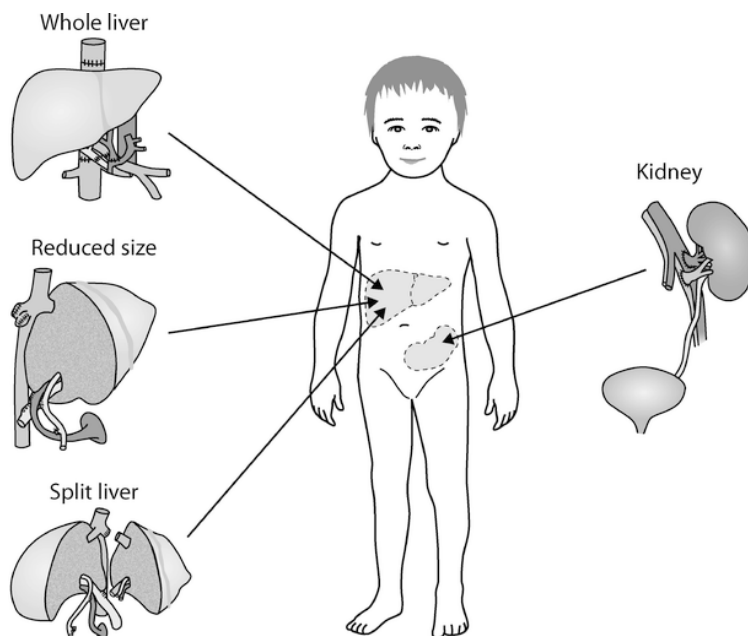


Figure 5. Schematic presentation of combined liver-kidney transplantation (CLKT). Whole livers or technical variant grafts (i.e. reduced or split) are also used as in LT. Reprinted with permission by Springer Nature from Jalanko H, Pakarinen M. Combined liver and kidney transplantation in children. *Pediatric Nephrology*. Springer Berlin Heidelberg. Copyright © IPNA 2013.

2.3.1 Indications

The reasons for CLKT vary between pediatric and adult patients. Two main indications in children are autosomal recessive polycystic kidney disease (ARPKD) and primary hyperoxaluria type I (PH-1). Other less common indications include HRS, drug-induced nephrotoxicity, methylmalonic acidemia and atypical hemolytic-uremic syndrome (104, 105).

ARPKD is a form of childhood ciliopathy with an incidence varying from 1:10,000 to 1:40,000 caused by mutations in the *PKHD1* gene (106). ARPKD mainly affects the kidneys and liver, leading to polycystic kidneys and congenital hepatic fibrosis (CHF), although the clinical features and prognosis are heterogeneous (106). According to a Finnish study, of 27 ARPKD patients who survived through the neonatal period 44% were not transplanted, 41% received isolated KT and 15% received CLKT (107).

In contrast, PH-1 is an autosomal recessive disease with a yearly incidence around 1:120,000 live births caused by mutations in the *AGXT* gene (108). In PH-1, an accumulation of oxalate and glyoxalate due to lack of a liver-specific enzyme (i.e. problem in the liver) leads to kidney stones, nephrocalcinosis and renal failure, although clinical features and prognosis are variable with a median age of 24 at the time of ESKD (108).

ARPKD accounts for approximately 70% of CLKT indications in some centers (109-111) while PH-1 accounts for 80% in some others (112). In the United States, the primary liver and kidney disease indications for pediatric CLKT are PH (37%) and CHF (18%), and oxalate nephropathy (36%) and polycystic kidney disease (25%), respectively (113).

In adults, hepatitis C virus (HCV) accounts for 30% to 37% of primary liver disease and HRS 22% to 58% of kidney disease etiology in CLKT patients (114-116). Hepatorenal syndrome (i.e. HRS) is a functional kidney disorder without structural damage associated with liver failure and is usually reversible with LT although renal impairment may not improve in some instances after LT, which is why CLKT may be needed (103). An overall incidence of HRS is 9% in adults undergoing LT, and life-expectancy is shorter compared to adults without HRS, but also compared to adult CLKT patients with HRS (116-117).

In adults, other liver and kidney indications for CLKT include non-alcoholic steatohepatitis, alcoholic liver disease, cryptogenic cirrhosis, diabetic nephropathy, CNI toxicity, chronic glomerulonephritis and polycystic kidney disease (114-116, 118).

2.3.2 Long-term outcomes

2.3.2.1 General

Since CLKT combines both LT and KT, short- and long-term complications are expected. Briefly, complications after CLKT include infections, bleeding, biliary complications, and urologic complications (119).

Liver graft is thought to provide an immunological safeguard to the kidney graft in CLKT since both grafts are from the same donor. Both pediatric and adult patients who receive CLKT experience less acute kidney rejections than patients who receive isolated KT (109, 110, 120-123) although some studies have proposed that this is due to more lenient immunosuppression in KT patients (124). Kidney grafts in KT patients have shorter half-life than kidney grafts in CLKT patients (124).

Based on selected literature, five-year patient survival ranges from 82% to 100% after pediatric CLKT (110, 111, 113, 125, 126) and 65% to 82% after adult CLKT (116, 120, 127) being 76% in the largest study with 3,127 CLKTs (127). However, both primary liver and kidney disease etiology affects these survival estimates (113, 115, 128). Patient survival for pediatric CLKT patients is shown in Figure 6.

As depicted in Figure 6, pediatric CLKT patients' survival is similar to that of pediatric LT patients but inferior to pediatric KT patients although some smaller studies indicate better survival after CLKT (124). Early post-transplantation mortality is higher for CLKT and LT patients compared to KT patients, which is explained at least partly by the technical complications related to the LT itself.

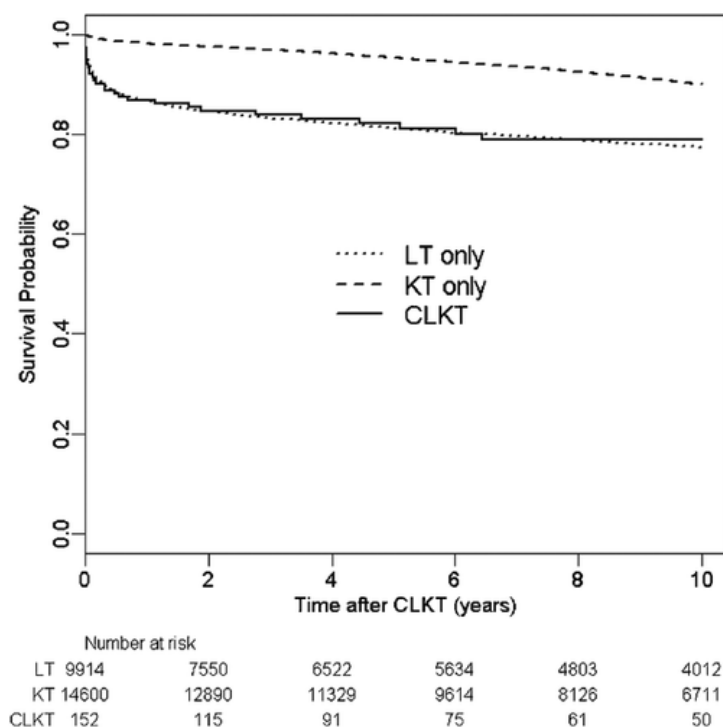


Figure 6. Patient survival after pediatric primary CLKT, primary KT, and primary LT in the United States for patients transplanted between October 1987 and February 2011.

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In adults, patients with CLKT seem to have better survival than LT patients (116, 127) even after excluding patients who died within 30 days after transplantation and adjusting for potential confounders including dialysis, MELD score and age (127). More recently, a survival benefit of four months in CLKT over LT patients who were not on dialysis was estimated although this was clinically deemed a marginal benefit (129).

In line with pediatric studies, adult KT patients seem to have better survival than CLKT patients (120, 123) albeit some studies suggest similar survival in these patients (122). Several factors, including unmeasured confounding, can explain these findings between and within these observational studies.

2.3.2.2 Renal function after CLKT

Relatively few studies have focused on long-term renal function after CLKT. Here, long-term can be defined as follow-up period beyond one year after transplantation, and therefore focus will be on these studies.

Renal function after pediatric CLKT declines over the years (109, 110) (Table 5). Nonetheless, relatively stable eGFR has been reported in a mixture of CLKT and sequential liver and kidney transplantation patients (130). In addition, other studies (112, 131) have reported eGFR only at last follow-up with a median follow-up of around 6 years (Table 5). None of these studies have used measurement of GFR.

Table 5. Renal function up to ten years after and at last follow-up after pediatric CLKT

Study (ref)	Patients, n ^a	eGFR (mL/min/1.73 m ²)		
		Year 1	Year 5	Year 10
Ranawaka (109)	40	68	59	50
Quintero Bernabeu (110)	14 ^b	83	73	70
		Last follow-up ^c		
Duclaux-Loras (112)	18	71		
Schmaeschke (131)	23	71 ^d		

a initial number of patients

b includes two sequential liver and kidney transplantations

c median follow-up ranged from 5.6 to 6.3 years

d calculated from individual GFRs given in article

Although primary diagnosis can affect renal function after CLKT, comparable renal function in PH-1 and ARPKD patients has been reported (112). In opposition to this, some studies show worse renal function in PH-1 patients (109, 131) but nonetheless, stable eGFR during a 10-year follow-up (109). In an earlier study from the same center, renal function improved more slowly in PH-1 patients than in patients with other indications, and most of the PH-1 patients show oxalate deposits in renal tubules after CLKT, which can influence later renal function (132).

Adult patients show relatively stable kidney function with eGFR around 50-60 mL/min/1.73 m² up to 5 years after CLKT (133). Again, primary diagnosis has an impact, and mean eGFR is around 30 and 40 mL/min/1.73 m² lower in patients with HCV compared to patients with other etiologies at one and five years after CLKT, respectively (134). However, less drastic differences between liver disease etiology and eGFR were observed in a registry-based study with 2,606 CLKT patients (115) (Table 6). For some liver disease groups, renal function remains steady or improves slightly, but in some it declines although eGFR remains stable across disease groups when pooled. Unfortunately, these types of large registry studies lack detailed information of factors that might influence renal function.

Table 6. Renal function up to 5 years after adult CLKT stratified by liver disease

Liver disease	eGFR (mL/min/1.73 m ²)		
	Year 1	Year 3	Year 5
Alcoholic liver disease	61	61	63
Alcoholic liver disease and HCV	64	65	63
Cryptogenic cirrhosis	57	54	55
Hepatitis B virus	60	59	59
Hepatocellular carcinoma	68	60	71
HCV	66	61	59
Non-alcoholic steatohepatitis	60	59	55
Primary biliary cirrhosis	58	63	59
Primary sclerosing cholangitis	61	54	56
Pooled eGFR ^a	62	60	59

Modified for ref 115.

^a calculated with R 3.1.1 using meta package

Only a handful of studies have compared renal function of CLKT patients to renal function of KT patients with a follow-up beyond one year after transplantation (109, 123). Pediatric CLKT patients have, on average, better renal function than age-, transplantation year- and immunosuppression-matched pediatric KT patients, albeit both transplantation groups have declining renal function during the follow-up (109). In a study with 204 patients, eGFR

was stable in CLKT and declined in KT patients, but more so in patients who were positive for donor-specific antibodies (DSA) at the time of transplantation (123). In addition, CLKT was associated with lower odds of a combined end-point of graft loss or GFR decline over 50% when all patients were analyzed together (123). Unfortunately, eGFR was not analyzed in a continuous manner in a multivariable regression (123) which would be interesting in the light of limited data in adults.

In contrast, continuously analyzed eGFR at one month associates with long-term eGFR in pediatric KT patients (135), which might also apply to pediatric CLKT patients.

2.4 Immunology and immunosuppression

The immune system is responsible for rejection of foreign material, including organ allografts, through various mechanisms, including cell- and antibody-mediated responses. Suppression of these responses, especially T-cell mediated responses, with immunosuppressive drugs is the core of transplantation medicine.

2.4.1 HLA-system and HLA-matching

The human leukocyte antigen (HLA) system is a set of polymorphic genes located at chromosome 6, which has a pivotal role in both innate and adaptive immunological responses in the human body (136, 137). These genes include class I (HLA-A, HLA-B, and HLA-C), and class II (HLA-DP, HLA-DQ, and HLA-DR) genes, which encode respective HLA class I and II molecules found in all somatic cells (class I) and in immune cells (class II). These HLA molecules (or HLA antigens) bind and present antigenic peptides (i.e. small particles from proteins) to T-cells to facilitate cellular-mediated immune responses, the central tenet of allograft rejection and immunosuppression in SOT, although according to humoral theory of transplantation, antibodies are the cause of rejections (138).

HLA-genes are greatly polymorphic with at least 13,023 alleles identified (137), which mitigates allograft recipient and donor HLA compatibility in organ transplantation. Generally,

HLA-A, -B and -DR antigens are compared before transplantation to determine HLA compatibility (i.e. HLA typing), and donor and recipient are said to be matched if no allele differences can be observed in these three HLAs on a scale from zero to six mismatches. Only a minority of KT patients are zero-mismatched.

Despite the fact that HLA compatibility is associated with better graft survival in KT patients (139,140), it has no clinical role in LTs. Some early studies highlighted the dual role of HLA compatibility in LT patients in that rejections occurred less often whereas graft failure occurred more often in HLA-matched patients (141). Nonetheless, more recent and larger studies have shown no association between HLA compatibility and five-year graft failure after adult and pediatric LT (142, 143).

2.4.2 Donor-specific HLA antibodies (DSA)

An allograft recipient can have circulating antibodies due to a prior sensitizing event, including previous transplantation, pregnancy and blood transfusion. These preformed antibodies can be directed against donor HLA antigens present in the allograft (i.e. donor-specific HLA antibodies; DSA) and are associated with antibody-mediated rejection, especially in kidney grafts (144-146).

Contrary to this, liver grafts seem to be more resilient to antibody-mediated rejection, including hyperacute rejection (147, 148), although a predictive score for chronic antibody-mediated rejection in LT setting has been developed (149). In general, screening for HLA antibodies before LT is recommended for risk classification (150).

More sensitive fluorescent-based solid-phase immunoassays, like single-antigen beads (SAB) technology, to detect HLA antibodies have replaced less sensitive and more cumbersome methods although in solid-phase immunoassays, like SAB, the threshold for positive cut-off for normalized mean fluorescence intensity (MFI) is not standardized and varies on a case-by-case basis (151, 152).

The prevalence of DSAs after pediatric LT is 41% ranging from 8% to 75% between studies (8). More recently, prevalence was 24% for DSAs and 23% to 56% for class II DSAs in pediatric LT patients (7, 153). In contrast, the prevalence of preformed and de novo DSAs (i.e.

formed after LT without evidence of preformed DSAs) varies from 5% to 22% and from 8% to 20% in adult LT patients, respectively (154-158).

In both pediatric and adult patients, DSAs are most often found to be class II (7, 156), and these can persist after LT, especially in patients with high MFIs (155). DSAs have also been associated with chronic rejection, portal inflammation, liver fibrosis and cirrhosis (7, 153, 159) but not inferior graft survival or acute rejections (7) in pediatric LT patients. Small sample sizes pose challenges for multivariable regression models to evaluate the impact of DSAs on different outcomes (160).

2.4.3 Immunosuppression

Much of the success of solid organ transplantations has depended on effective immunosuppressive medications for treatment of allograft rejections resulting from activation of a series of immunological acts initiated by different cells, including T-lymphocytes (T-cells). Optimal use of immunosuppressive drugs requires a subtle balance between maximizing benefits and minimizing harms, especially in pediatric patients in whom cumulative doses of these drugs are higher due to life-long exposure. Detailed description of immunosuppressive drugs and adverse effects are provided elsewhere (161-163).

Briefly, cyclosporine (CsA) and tacrolimus (TAC) are calcineurin inhibitors (i.e. CNIs), which hinder the activity of calcium-dependent phosphatase calcineurin. This, on the other hand, leads via additional mechanisms to hampering of T-cell activation and proliferation (161, 162). Either CsA or TAC is combined with one of the purine synthesis inhibitors [like azathioprine (Aza) and mycophenolate mofetil (MMF)] and/or corticosteroids [like methylprednisolone (MP)] for a triple or dual immunosuppression regimen. Other immunosuppressive drugs include rapamycin (mTOR) inhibitors (like sirolimus) and monoclonal antibodies (like basiliximab) (161, 162). The typical mechanism of these drugs is to suppress the function of T-cells in one way or another, commonly via cytokines, including interleukin-2.

The therapeutic dose of CNIs for any given patient is adjusted according to blood trough levels. Therapeutic blood levels range from 150 to 350 µg/L and 5 to 20 µg/L for CsA and TAC, respectively (164). Nonetheless, there can be variation in target blood trough levels

as well as different immunosuppression regimens between transplant centers although guidelines highlight some specific aspects for long-term immunosuppression, including target trough levels for TAC and corticosteroid withdrawal (97).

In Finland, the initial triple immunosuppression regimen for pediatric LT patients includes CsA, Aza and MP. In addition, basiliximab is given as an induction immunosuppression. In contrast, in the United States, the most commonly used initial immunosuppression regimen in pediatric LT patients consists of TAC, mycophenolate and corticosteroids, although around one-fifth of patients are on corticosteroid-free regimen, and fifty-seven percent of patients do not receive induction immunosuppression (27).

Immunosuppressive drugs have various adverse effects, which can be classified to immunosuppression-related, such as infection and malignancy, and drug-specific, such as post-transplant diabetes mellitus with the use of corticosteroids, nephrotoxicity with the use of CNIs, and bone-marrow suppression with the use of Aza (163).

A limited number of RCTs in pediatric patients have been published considering superiority of CsA- or TAC-based immunosuppression regimen. One study with children and one with a mixed population of adults and children were included in the Cochrane review published in 2006 for comparisons between CsA- and TAC-based regimens after LT (165). Pooling these two studies showed inconclusive results considering patient mortality and graft loss but supported TAC over CsA in acute rejections and withdrawals (165).

3. Aims of the study

Liver transplantation effectively improves or saves a patient's life in severe liver diseases. However, long-term sequelae include many aspects that might influence later health. In this thesis, long-term issues after pediatric liver transplantation were studied, including liver histology and radiological imaging, immunological assessment and longitudinal renal function. In addition, long-term renal function after combined liver and kidney transplantation in both pediatric and adult patients was studied.

The aims of the study were to appraise

- 1) prevalence of late hepatic artery thrombosis after pediatric LT (I)
- 2) prevalence of donor-specific HLA antibodies and their association with liver histology after pediatric LT (II)
- 3) longitudinal renal function after pediatric LT (III)
- 4) longitudinal renal function after pediatric and adult CLKT (IV)

4. Patients and methods

4.1 Patients and study design

The details of patients and study design are shown in Table 7. All patients were operated at Helsinki University Central Hospital either in Children's Hospital (pediatric unit) or in Transplantation and Liver Surgery Clinic (adult unit).

Table 7. Characteristics of included studies

	Study I	Study II	Study III	Study IV
Design	C	C	L	L
Primary focus	HAT	DSA	GFR	GFR
Transplantation era	1987-2007	1987-2007	1987-2007	1993-2011
Transplantation type	LT	LT	LT	CLKT
Number of patients	34	50	57	34 ^a
Age limit for pediatric patient	18	18	18	16
Median age at LT, yrs	1.7	2.6	3.3	2.5/44.6 ^b
Number of control patients	-	-	-	96 ^c

a includes 11 pediatric and 23 adult patients.

b age for pediatric and adult patients, respectively.

c includes 27 pediatric and 69 adult KT patients

CLKT; combined liver-kidney transplantation, C; cross-sectional, DSA; donor-specific HLA antibodies, GFR; glomerular filtration rate, HAT; hepatic artery thrombosis, KT; kidney transplantation, L; longitudinal, LT; liver transplantation

The patient selection process in studies I and II is shown in Figure 7. Of 66 potential patients, 52% (34/66) (I) and 76% (50/66) (II) were included. Patients who were not included were older and received more often whole liver grafts compared to included patients. In study III, the focus was on patients operated at Children's Hospital; some pediatric patients operated in the adult unit were therefore not included. The same 32 patients were included in both I and II.

In cross-sectional studies, MRI (I) or blood sample for DSA analysis (II) was taken once for each study patient as part of a study protocol between 2009 and 2011. In study I, pre-, peri- and postoperative risk factors were retrospectively collected and contrasted to MRI findings (Table 8).

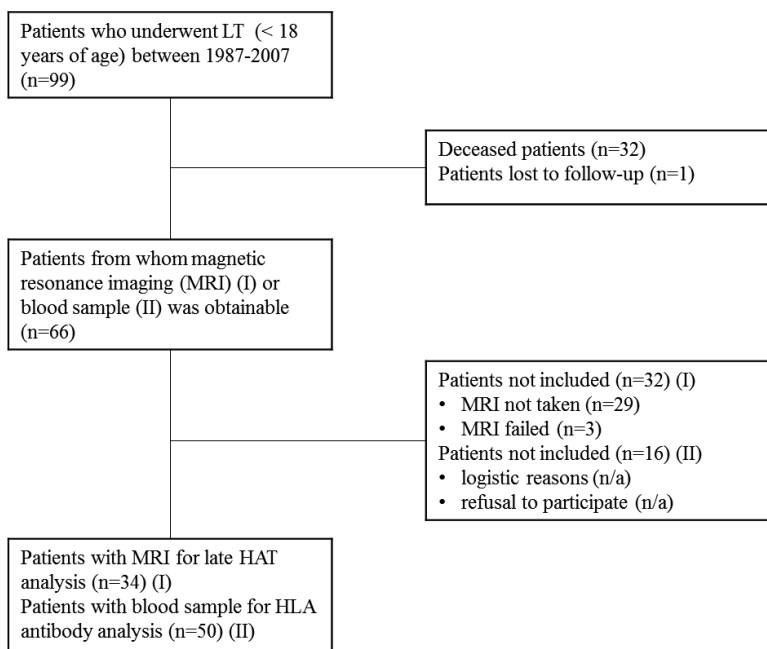


Figure 7. Flow chart of patient selection process in Studies I and II.

Table 8. Pre-, peri- and postoperative factors investigated in Study I

Preoperative laboratory values

Hemoglobin
Hematocrit
Platelet count
Thromboplastin time
Activated partial thromboplastin time
Factor V

Perioperative variables

Donor-recipient weight ratio
Donor-recipient age ratio
Frozen plasma used at LT
Platelets used at LT
Blood loss at LT
Cold ischemia time
Anhepatic phase

Postoperative variables

Length of stay in intensive care
Low-molecular weight heparin (LMWH) duration
Acetylic salicylic acid (ASA) duration
Rejection episodes
Cytomegalovirus viremia
Biliary complications

Kidney transplantation patients were selected as matched controls for CLKT patients (10 pediatric and 23 adult CLKT patients) (IV). CLKT and KT patients were matched regarding gender, age (± 2 years), and transplantation year (± 2 years) (IV).

Data were collected from the patients' medical records in all studies, and from the national LT and transplantation registry (I, II, IV). Additionally, the Finnish Red Cross Blood Service's clinical laboratory database was utilized (II).

4.2 Immunosuppression

Patients used initially cyclosporine A (CsA) based triple immunosuppression with Aza and MP. If clinically indicated, CsA was replaced by TAC and Aza by MMF, which was also used in adults since 2006. MP dose was tapered to 0.25 mg/kg/day and was switched to every

other day usage at 6 months with the aim of discontinuation when the patient reached adulthood or within 12 months in adults except in the case of autoimmune liver diseases. Since 1999, basiliximab was used as an induction therapy in pediatric patients during LT and on the fourth postoperative day.

4.3 Imaging (I)

Patients underwent abdominal MRI with a 1.5-T magnetic resonance scanner (Achieva, Philips, Best, the Netherlands) using gadolinium-based contrast agent. Small children underwent MRI under general anesthesia. A pediatric radiologist unaware of the patients' clinical status re-evaluated the MRI images and Doppler US reports.

4.4 HLA antibodies (II)

Serum samples were screened and identified for HLA class I (-A, -B, and -C) and class II (-DR and -DQ) antibodies with bead-based commercial immunoassays (One Lambda LabScreen® Mixed and LabScreen® Single) using HLA Fusion Software (One Lambda Inc., Canoga Park, CA). Mean Fluorescence Intensity (MFI) value of 1,000 was considered positive. HLA antibodies were analyzed unaware of patients' information.

4.5 Liver histology (I, II)

Liver biopsy specimens were fixed, embedded and stained using routine histochemical staining. Additionally, specimens were immunostained for complement component 4d (C4d) deposits using polyclonal rabbit anti-human antibody (Cat.no BI-RC4D, Biomedica, Vienna, Austria) (I) and for cytokeratin-7 (CK7) using SP52 monoclonal antibody and Ultra-View Universal DAB Detection Kit (Vent Ana, Tucson, Arizona, USA).

Two liver pathologists re-evaluated the biopsy specimens unaware of patients' clinical information with the use of the following semiquantitative scoring structure: CK7 for periportal hepatocytes (0-3), portal inflammation (0-3), fibrosis (0-4) and bile duct proliferation (0-2). Ten percent cut-off for C4d deposits in microvascular endothelium was used.

4.6 Renal histology (III)

Renal biopsy specimens were fixed, embedded and stained using routine histochemical stainings, and re-evaluated by a nephropathologist unaware of patients' clinical information. Specimens were deemed representative if ten or more glomeruli or marginally representative if one to nine glomeruli were present. The following CNI-induced histological changes were registered: 1) isometric vacuolization of tubules, 2) medial and adventitial hyaline deposits, 3) intimal fibrosis/onion skinning of arterioles, 4) splitting of glomerular basement membranes, and 5) focal glomerulosclerosis (166). These changes were coded as no changes, mild, moderate, and severe changes.

4.7 Renal function (III, IV)

Glomerular filtration rate was measured with plasma clearance of ^{51}Cr -EDTA as part of routine clinical follow-up in pediatric patients, and cross-sectionally in a subgroup of adult CLKT patients (IV). Measured GFRs taken before 2006 were corrected with the use of Bröchner-Mortensen equation (16) to facilitate comparable mGFRs taken before and after 2006. EDTA-clearance distribution volumes $< 15\%$ or $> 35\%$ were excluded (III).

In study III, estimation of GFR was also made with the Schwartz (167), Counahan-Barratt (168) and cystatin C and urea based CKiD III (20) equations, and in Study IV, with bedside Schwartz (pediatric) (20) and CKi-EPI equations (adults) (19). GFRs are shown with unit of mL/min/1.73 m² (III, IV) and mGFRs also without BSA correction (i.e. mL/min) using Haycock formula (169) (III).

4.8 Statistics

Statistical analyses were performed with PASW version 17 (IBM, SPSS Inc. Chicago, IL) (III), IBM SPSS version 22 (IBM, Armonk, NY) (III), Stata 12.1 (StataCorp LP, College Station, TX) (I, II, IV), JMP Pro 10.0.02 (SAS Institute, Cary, NC) (II), SAS 9.3 (SAS Institute, Cary, NC) (I), and R 3.1.1 (The R Foundation for Statistical Computing, Vienna, Austria) (II, IV). Several

statistical methods were used throughout, but some less common methods are detailed here.

Continuous variables are shown with means and standard deviation (SD) or with medians and interquartile ranges (IQR). Comparison of medians between two independent groups was made with the Bonett-Price method with 95% confidence interval (CI) for difference in medians (170) (I, III, IV). An exact logistic regression was used to study association between late HAT and surgical factors (i.e. graft type and anastomosis type) (I) and type of transplantation and immunosuppression (II). In addition, penalized logistic regression (171) was used (II). In study I, P-values were also adjusted for due to several comparisons made (172). In study II, CIs for unadjusted prevalence ratios (PR) and odds ratios were calculated with recommended methods more suitable for small sample size (173). In study IV, linear mixed models were used for longitudinal GFR comparison between adult CLKT and matched adult KT patients so that matched factors were adjusted for in addition to group and follow-up time.

4.9 Ethical issues

These studies were studies or part of studies approved by the Ethics Committee for Pediatrics, Adolescent Medicine, and Psychiatry and Ethics Committee for Surgery of the Hospital District of Helsinki and Uusimaa (application numbers 159/E7/2005, 345/13/03/2008, and 268/13/03/02/2010). Informed consent was obtained from patients, or in the case of minors, also from parents or guardians.

5. Results

5.1 Patient survival after LT and CLKT

Patient survival for 90 pediatric patients who received deceased donor liver grafts between 1987 and 2007 at Helsinki University Central Hospital is shown in Figure 8. One-, five-, ten-, and twenty-year patient survival (95% CI) was 82% (73% to 89%), 74% (64% to 82%), 69% (58% to 78%), and 59% (47% to 69%), respectively.

Patient survival for 11 pediatric CLKT patients operated between 1999 and 2011 is shown in Figure 9. One-, five- and ten-year patient survival (95% CI) was 91% (51% to 99%), 91% (51% to 99%), and 81% (42% to 95%), respectively.

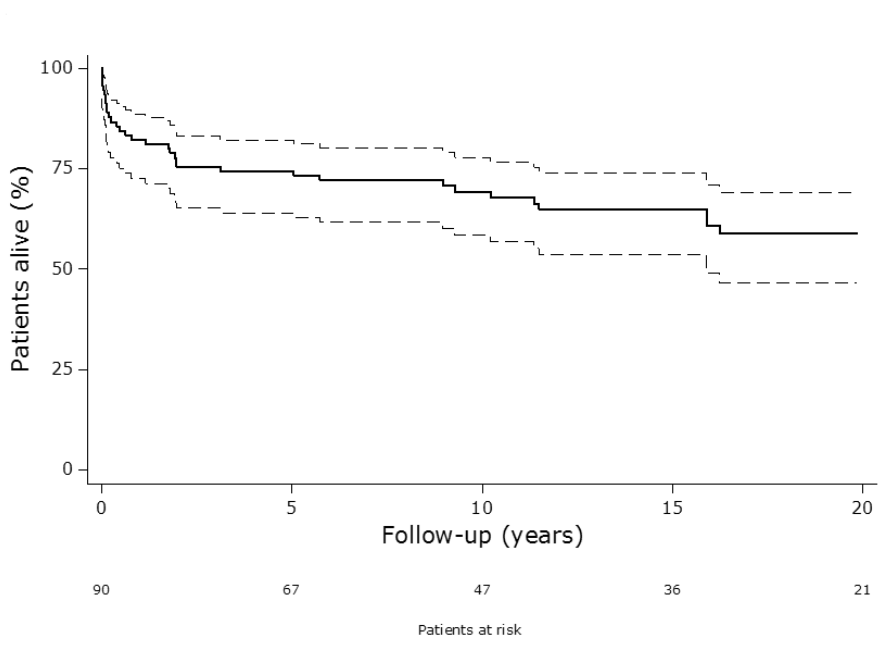


Figure 8. Kaplan-Meier plot for patient survival (solid line) with 95% confidence interval (dashed line) in 90 pediatric LT patients operated from 1987 to 2007. Seven combined and one sequential liver-kidney transplantations excluded (not previously published).

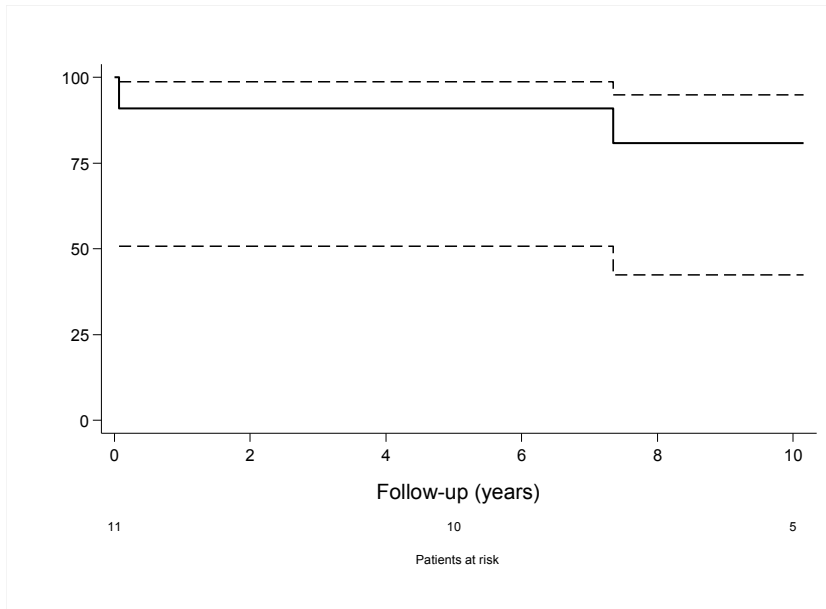


Figure 9. Kaplan-Meier plot for patient survival (solid line) with 95% confidence interval (dashed line) in 11 pediatric CLKT patients operated between 1999 and 2011.

The respective survival for 23 adult CLKT patients operated between 1993 and 2011 was 100% at one, 96% (73% to 99%) at five and 70% (44% to 85%) at ten years after transplantation [Figure 1 in (IV)].

5.2 Late HAT (I)

The final population for the HAT study consisted of 34 patients who underwent MRI with a median follow-up of 9.5 years after LT (Table 9). Forty-one percent had biliary atresia as primary indication, and metabolic diseases were mostly tyrosinemia (57%). Three of the 34 patients received CLKT, and three received re-grafts from 4 to 20 years before the study MRI evaluation. The reasons for graft failure in these three re-transplant patients were primary non-function, HAT and chronic rejection.

Table 9. Selected characteristics for 34 patients who underwent MRI

Gender, n (%)	
Male	21 (62)
Female	13 (38)
Diagnosis, n (%)	
BA	14 (41)
MD	7 (21)
Hepatitis	7 (21)
Other	6 (18)
Liver graft type, n (%)	
Whole	8 (24)
Reduced	26 (76)
Arterial anastomosis, (%)	
Hepatic artery	16 (47)
Aorta	17 (50)
Not available	1 (3)
Mean (SD) age at time of MRI, yrs	15.4 (7.0) ^a
Median (IQR) follow-up time, yrs	9.5 (4.0 to 16.4) ^b

a median age 16.5 (9.9 to 19.2) years (not reported originally)

b mean follow-up 10.2 (6.3) years (not reported originally)

BA; biliary atresia, MD; metabolic diseases, MRI; magnetic resonance imaging

Late hepatic artery thrombosis was diagnosed with abdominal MRI in 44% (95% CI 29% to 61%) of 34 patients (n=15). In three of these patients, diagnosis of HAT was made with an angiography preceding MRI.

Ultrasonography correctly diagnosed late HAT in six patients, yielding sensitivity of 40% (95% CI 20% to 64%). Interestingly, collaterals were visible at least to some extent in four-fifths of the thrombosis patients.

Late HAT patients were transplanted more often with reduced sized liver grafts than patients without HAT (93% versus 63%), and hepatic artery anastomosis was done more often to the aorta in patients with than without HAT (73% versus 33%). These two surgery-related

factors were simultaneously adjusted for late HAT (Table 10). Both reduced graft and hepatic artery anastomosis to the aorta was associated with increased odds of late HAT, albeit confidence intervals included the possibility for decreased odds of late HAT.

Table 10. Results for late HAT adjusted with graft and artery anastomosis type

Odds ratio (95% CI) for late HAT	
Graft type	
Reduced	5.8 (0.5 to 308.1)
Whole liver (reference)	
Type of arterial anastomosis	
Aorta	3.9 (0.7 to 26.2)
Hepatic artery (reference)	

Based on exact logistic regression due to small sample size. HAT; hepatic artery thrombosis.

Based on a vast literature of risk factors associated or not associated with HAT, several pre-, peri- and postoperative factors were also analyzed between patients with and without late HAT (Table 8). Of these 19 factors, donor-recipient weight and donor-recipient age ratios were higher and duration of ASA treatment was shorter in patients with than without HAT (Table 11). The respective P-values were 0.03, 0.15 and 0.15 after multiplicity adjustment. More detailed results in [Table 5 in Study (I)]. Donor weights were similar in both no late and late HAT patient groups (median 60 vs. 60 kg) but donor ages were higher in the late HAT patient group (median 36 vs. 17 years).

Table 11. Differences in peri- and postoperative factors between HAT groups

Variable	No late HAT	Late HAT	Difference (95% CI)
D-R weigh ratio	1.9 (1.1 to 4.7)	5.4 (3.1 to 6.4)	-3.5 (-5.5 to -1.4)
D-R age ratio	3.4 (1.2 to 10.9)	21.7 (7.5 to 36.2)	-18.3 (-32.1 to -4.5)
Duration of ASA, days	1,201 (153 to 1,454)	128 (46 to 1,196)	1,073 (271 to 1,865)

Values are medians (IQRs) and difference in medians between no late HAT (n=19) and late HAT (n=15) patients. CIs not adjusted for multiple comparisons. Thirty patients used ASA (n=14 no late HAT and 16 late HAT).

ASA; acetylic salicylic acid, D-R; donor-recipient, HAT; hepatic artery thrombosis

Forty-three percent (6/14) of the HAT patients with a biopsy specimen available had at least some chronic cholestasis compared to 13% (2/16) of no late HAT patients with biopsy specimen, although based on a handful of patients. No drastic differences were observed between the selected laboratory values shown in Table 12 [Table 3 in (I)].

Table 12. Biochemical variables for patients with late HAT and without late HAT

Laboratory test	no late HAT (n=19)	late HAT (n=15)
Alanine aminotransferase (U/L)	20 (14 to 30)	29 (16 to 35)
Gamma-glutamyltransferase (U/L)	16 (13 to 33)	34 (22 to 59)
Alkaline phosphatase (U/L)	182 (79 to 233)	149 (107 to 288)
Total bilirubin (μmol/L)	13 (8 to 15)	9 (7 to 19)
Bile acids (μmol/L)	4.4 (3.3 to 11.2)	9.2 (4.2 to 16.8)
Thromboplastin time (%)	102 (80 to 112)	107 (91 to 134)

Values are medians and interquartile ranges. Reference range for thromboplastin time 70% to 130%.

5.3 DSA (II)

Blood samples for DSA analysis were obtained from 50 patients with a median follow-up of 10 years after LT (Table 13). Most patients were on cyclosporine-based immunosuppression at the time of the blood sample drawn for analysis.

Sixty-six percent (n=33) of the analyzed patients had HLA antibodies, and 26 of these (79%) had DSAs. In other words, 52% (95% CI 39% to 65%) of 50 studied patients had DSAs, and these were most often (85%; n=22) against class II HLA antigens. Antibodies were typically against HLA-DQ locus (73% -DQ, 12% -A, 12% -DR, 4% -B). Median MFI (mean fluorescence intensity) for class I and class II DSAs is shown in Figure 10. Mean MFIs were 4,236 and 13,481 for class I and class II DSAs, respectively.

Table 13. Selected characteristics for 50 patients with blood samples for DSA analysis

Gender, n (%)	
Male	26 (52)
Female	24 (48)
Diagnosis, n (%)	
Biliary atresia	17 (34)
Metabolic disease	10 (20)
Hepatitis	6 (12)
Malignancy	6 (12)
Other	11 (22)
Transplantation type, n (%)	
Liver transplantation	42 (84)
Combined liver-kidney transplantation	8 (16)
CNI immunosuppression at DSA, n (%)	
Cyclosporine	35 (70)
Tacrolimus	13 (26)
No CNI	2 (4)
Mean (SD) age at the time of DSA, yrs	15.8 (7.9)
Median (IQR) follow-up time at DSA, yrs	10.0 (4.0 to 16.4)

CNI; calcineurin inhibitor, DSA; donor-specific antibodies

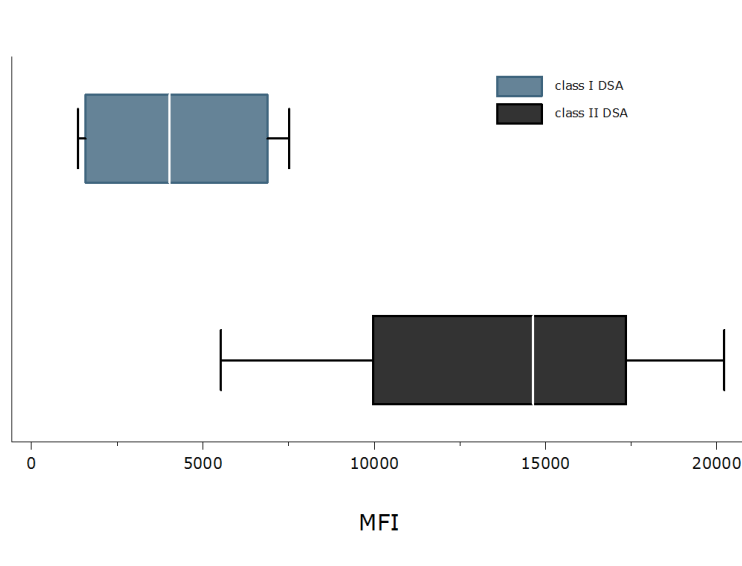


Figure 10. MFI for class I and class II DSAs. Median of 4,033 and 14,647 for class I and class II DSAs, respectively.

Follow-up time was longer in patients with compared to without DSAs at the time of blood sample drawn for analysis (median 5.6 versus 11.3 years). In addition, DSA-positive patients were more often LT than CLKT patients compared to DSA-negative patients (96% versus 71%).

Unadjusted prevalence ratios and odds ratios for DSA-positivity were calculated with different factors. Two factors with the strongest associations are shown in Table 14 [Table 3 in Study (II)]. As shown in Table 14, being a LT patient was associated with around five-fold higher prevalence for having DSAs compared to being a CLKT patient, and two-fold higher prevalence with use of cyclosporine instead of tacrolimus at the time of blood sample draw. These two factors were further adjusted simultaneously with the use of exact logistic regression suitable for small samples (lower part of Table 14). Nonetheless, only one CLKT patient was DSA-positive.

Table 14. Two factors with the strongest associations for DSA-positivity

	Odds ratio (95% CI)	PR (95% CI)
Transplantation type		
LT	10.3 (1.5 to 119.8)	4.8 (1.2 to 26.9)
CLKT (reference)		
CNI immunosuppression at DSA		
Cyclosporine	3.4 (0.9 to 11.2)	2.0 (0.95 to 4.9)
Tacrolimus (reference)		
<hr/>		
	Adjusted odds ratio (95% CI)	
Transplantation type		
LT	6.1 (0.6 to 312)	
CLKT (reference)		
CNI immunosuppression at DSA		
Cyclosporine	2.5 (0.5 to 14.2)	
Tacrolimus (reference)		

Prevalence ratio = risk ratio. Adjusted odds ratios based on exact logistic regression (not originally in Study II). CNI; calcineurin inhibitor, DSA; donor-specific antibodies; PR; prevalence ratio

As pointed out previously, follow-up time differed between DSA-positive and -negative patients. Recently, one study (156) highlighted that longer time-period between LT and DSA evaluation was associated with increased hazard for de novo DSAs; therefore, follow-up time was also analyzed in further analysis from which CLKT patients were excluded. Table 15 depicts results for DSA-positivity when follow-up time (model 1) and age at time of LT (model 2) was adjusted in 42 LT patients. An association for every year of follow-up and 1.8-fold higher odds for having DSAs was noticed; this association weakened after accounting for age at the time of LT.

Table 15. Logistic regression for DSA-positivity in 42 liver transplantation patients

	Odds ratio (95% CI)
Model 1	
Follow-up time, yrs	1.81 (1.17 to 21.6)
Model 2	
Follow-up time, yrs	1.04 (0.79 to 17.1)
Age at liver transplantation, yrs	0.88 (0.50 to 2.36)

Based on penalized logistic regression [please see (171)] with R 3.1.1 (not included originally in Study II).

DSA; donor-specific antibodies.

Liver histology specimens were available from 46 or 47 patients. Portal inflammation was more severe in patients with than without DSAs ($P=0.009$; exact Cochrane-Armitage Trend test) (Figure 11). Linear trends in other three histology parameters were less evident (P -values 0.195, 0.582, and 0.638 for fibrosis, CK7 for periportal hepatocytes, and bile duct proliferation, respectively).

Histology parameters were also dichotomized for calculation of unadjusted prevalence ratios with 95% CIs. Table 16 shows these ratios for each histology parameter in DSA-positive compared to DSA-negative patients. DSA-positive patients had around 9-fold higher prevalence of portal inflammation compared to DSA-negative patients although only one DSA-negative patient had portal inflammation, which led to wide CIs.

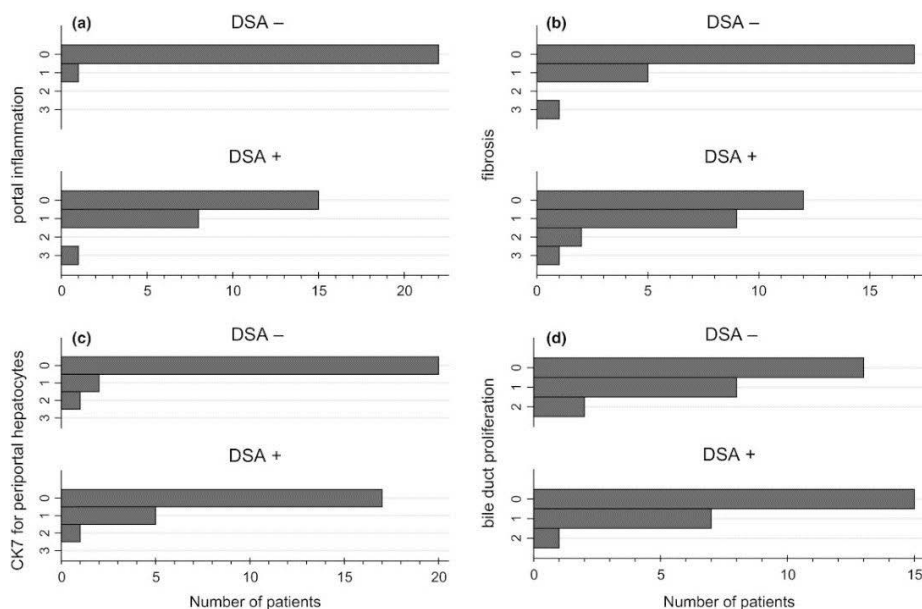


Figure 11. Liver histology findings in DSA-negative (DSA –) and DSA-positive (DSA +) patients considering (a) portal inflammation, (b) fibrosis (c) periportal hepatocytes, and (d) bile duct proliferation. Semiquantitative grading classification is shown in y-axis and number of patients in each category in x-axis.

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Table 16. Prevalence ratios for histology outcomes in DSA + and DSA - patients

Histology parameter	DSA + (n/N)	DSA - (n/N)	PR (95% CI)
Portal inflammation	9/24	1/23	8.6 (1.6 to 50.9)
Fibrosis	12/24	6/23	1.9 (0.9 to 4.3)
CK7 for periportal hepatocytes	6/23	3/23	2.0 (0.6 to 6.7)
Bile duct proliferation	8/23	10/23	0.8 (0.4 to 1.6)

CI; confidence interval, CK7; cytokeratin 7, DSA; donor-specific antibodies, PR; prevalence ratio

5.4 Renal function (III, IV)

Fifty-seven pediatric LT and 11 CLKT patients were studied in Study III and IV, respectively (Table 17). In addition, renal function of 23 adult CLKT patients operated between 1993 and 2011 were included [see Table 1 in (IV) for baseline characteristics]. Of these 23 adults, eleven were male and six had polycystic kidney disease. Median follow-up was 6.6 years from CLKT to cross-sectionally taken mGFR.

In Study III, CLKT patients were excluded altogether. One pediatric CLKT patient died shortly after transplantation; therefore renal function was studied for 10 CLKT patients operated between 1999 and 2011 (IV).

Table 17. Characteristics of 57 pediatric LT and 11 pediatric CLKT patients

	Study III	Study IV
Gender, n (%)		
Male	31 (54)	4 (36)
Female	26 (46)	7 (64)
Diagnosis, n (%)		
Biliary atresia	22 (39)	-
Metabolic disease	13 (23)	3 (27)
Hepatic malignancy	8 (14)	-
Acute liver failure	7 (12)	-
ARPKD	-	7 (64)
Other	7 (12)	1 (9)
Median (IQR) follow-up, yrs	11.0 (4.0 to 16.1)	9.0 (7.5 to 11.7)

ARPKD; autosomal recessive polycystic kidney disease, IQR; interquartile range, LT; liver transplantation, CLKT; combined liver-kidney transplantation.

5.4.1 Renal function after pediatric LT (III)

Renal function declined initially after transplantation, improved thereafter, and remained stable for up to five years (Figure 12). Mean (SD) measured GFR was 88 (27) before and 66 (21) at 15 years after LT. Thirteen percent of patients (4/31; n=3 G3a and n=1 G3b; see Table 3) and 31% (5/16; n=3 G3a and n=2 G3b) had a GFR below 60 mL/min/1.73 m² at five

and at ten years after LT, respectively. Biliary atresia patients had, on average, better GFR than patients with other diagnoses at one year after LT (96 vs. 72; 95% CI for mean difference 8 to 39).

Absolute mGFR (mL/min) increased in patients who were younger than five years of age at the time of LT, contrary to what was observed in mGFR with BSA correction (Figure 13). Mean (SD) absolute mGFR was 31 (9) and 59 (23) at one and 15 years after LT, respectively.

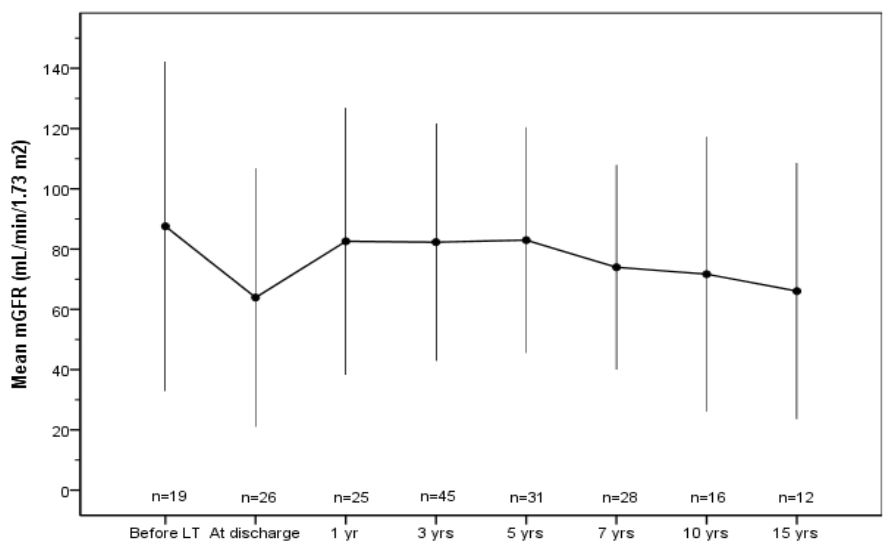


Figure 12. Evolution of mean (2SD) measured GFR (mL/min/1.73 m²) up to 15 years after pediatric liver transplantation. Figure modified from originally reported [see Figure 1 in (III)].

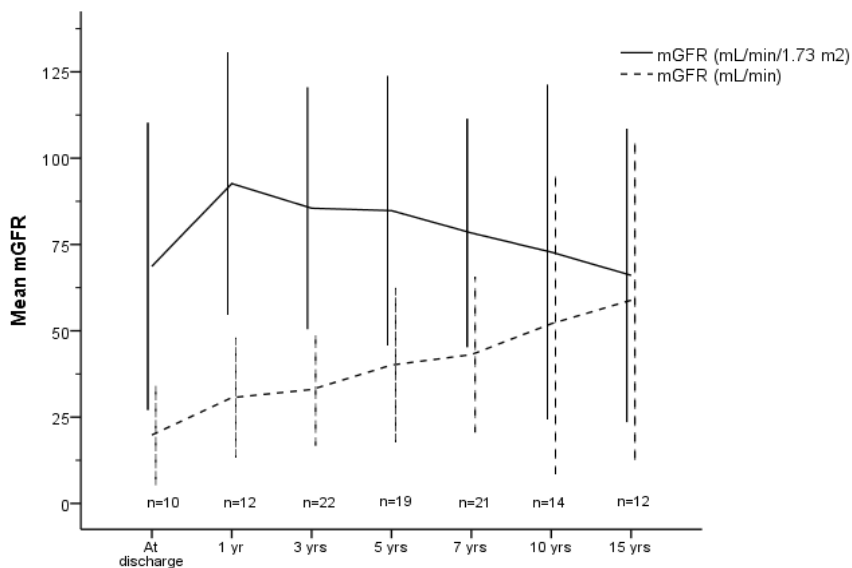


Figure 13. Mean (2SD) measured GFR with (solid) and without (dashed) body-surface area (BSA) correction in patients who were under five years of age at the time of LT. Figure modified from originally reported [see Figure 2 in (III)].

Measured GFR was, on average, overestimated by the three estimation equations (Figure 14). The creatinine-based equations, Schwartz and Counahan-Barratt, overestimated mGFR more than cystatin C and urea-based CKiD III equation [referred to as updated Schwartz in (III)]. In contrast, mGFR was, on average, higher than with cystatin C and urea-based equation at one year after LT (88 vs. 82; 95% CI for difference -4 to 15). However, as cystatin C has only been measured since 2003 therefore it was not available for all patients.

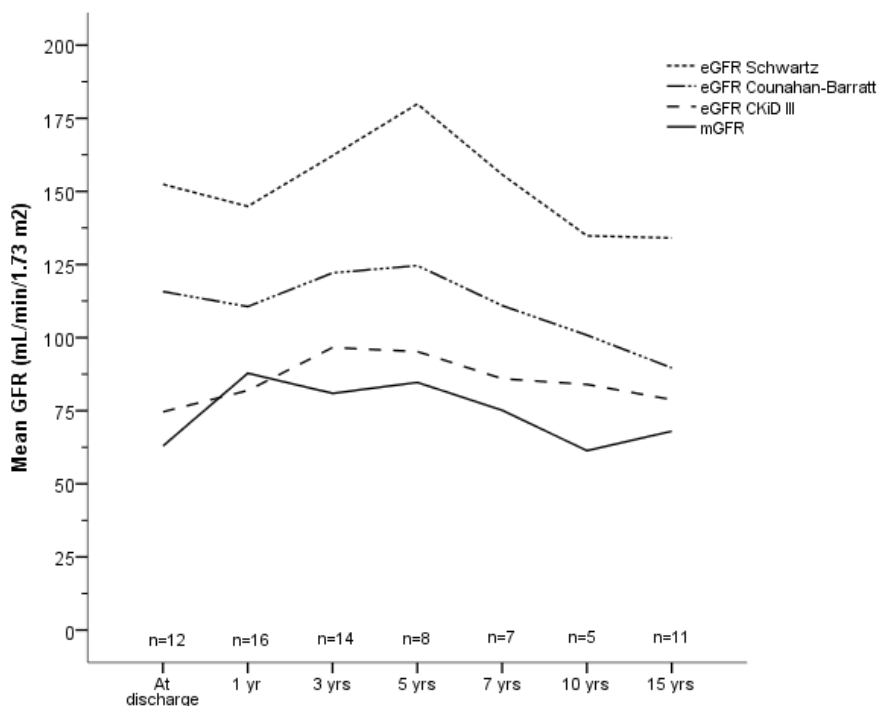


Figure 14. Comparison of mean GFR up to 15 years after LT with the use of three estimation equations and measured GFR (mGFR) based on plasma clearance of ^{51}Cr -EDTA. Figure modified from originally reported [see Figure 3 in (III)].

Seventy-six renal biopsies from 36 patients were analyzed. Histologic changes were evident in fifteen biopsies taken from 11 patients. Most of these patients had a metabolic disease as primary diagnosis. However, most (80%) of the histologic changes were mild and no severe changes were observed. The most common CNi-induced change was isometric vacuolization in the tubules. Glomerular filtration rate was lower in patients with compared to patients without histologic changes.

5.4.2 Renal function after CLKT (IV)

Renal function remained stable in pediatric CLKT patients during follow-up, albeit based on a small number of patients (Figure 15). Mean (SD) mGFR was 63 (17) at one and 70 (17) at

ten years after CLKT. In a pairwise comparison, mean GFR remained at a stable level of 66 in four patients from one to ten years after CLKT. Mean (SD) mGFR at last follow-up in ten pediatric patients was 60 (12).

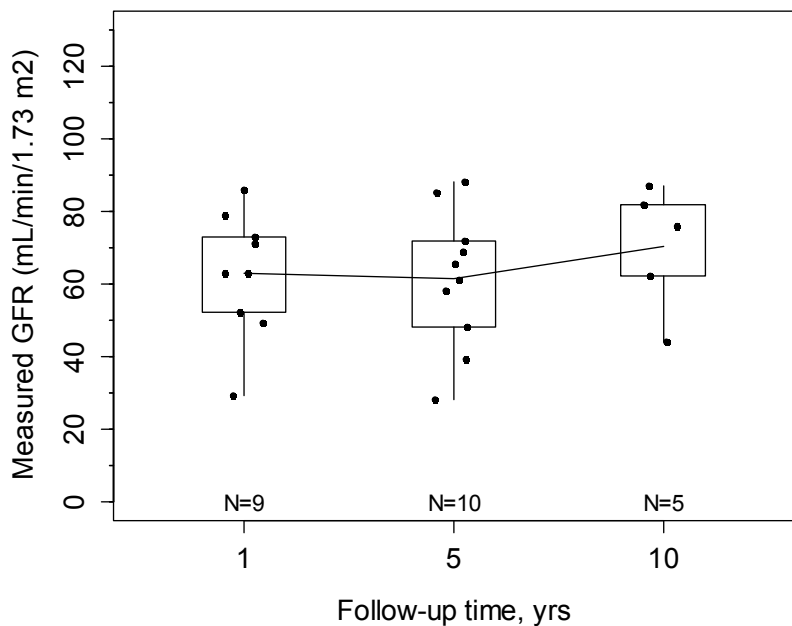


Figure 15. Measured GFRs between one and ten years after pediatric CLKT. Solid line indicates mean mGFR.

On the contrary, renal function declined in adult CLKT patients between one and ten years after transplantation (Figure 16). Mean (SD) estimated GFR was 75 (26) at one and 50 (16) at ten years after CLKT. In a pairwise comparisons with seven patients, mean (SD) eGFR declined from 66 (24) to 51 (18) (95% CI for difference 5 to 25). Measured GFR was taken once during the follow-up from a subsample of adult CLKT patients with a mean follow-up of 6.7 years. In these 12 patients, mean (SD) mGFR was 54 (22).

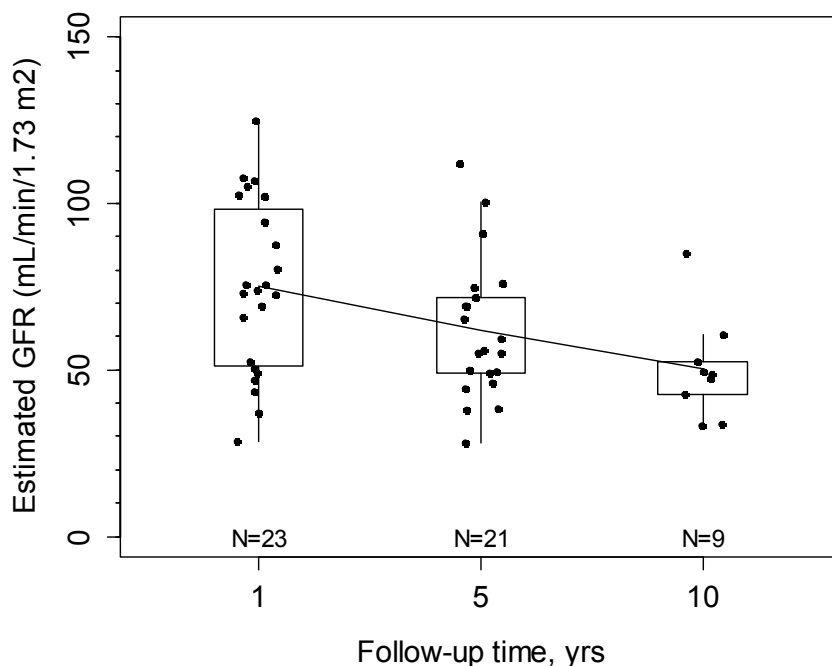


Figure 16. Estimated GFR between one and ten years after adult CLKT. Solid line indicates mean eGFR.

As in pediatric LT patients, the use of GFR estimation equations overestimated measured GFR. Overestimation of mGFR was, on average, 14 mL/min/1.73 m² in pediatric CLKT patients (n=10) with the bedside Schwartz equation and 11 mL/min/1.73 m² in adult CLKT patients (n=12) with the CKD-EPI equation [Figure 4 in (IV)]. In pediatric patients, the cystatin C and urea-based CKiD III equation overestimated mGFR less with a mean (SD) of 5 (6) mL/min/1.73 m² (Figure 17). Nine of these eGFRs were within mGFR interval of $\pm 30\%$, reflecting accuracy of 90% (95% CI 60% to 98%) [not included in (IV)].

Renal function of CLKT patients was contrasted to renal function of kidney transplantation patients. Twenty-seven pediatric KT patients were selected for ten pediatric and 69 adult KT patients for 23 adult CLKT patients as matched controls. The details of the control patients' characteristics are shown in [Table 2 in (IV)].

Glomerular filtration rate was, on average, better in pediatric CLKT patients compared to pediatric KT patients (Table 18). Both in adult CLKT and KT patients, renal function declined during the follow-up.

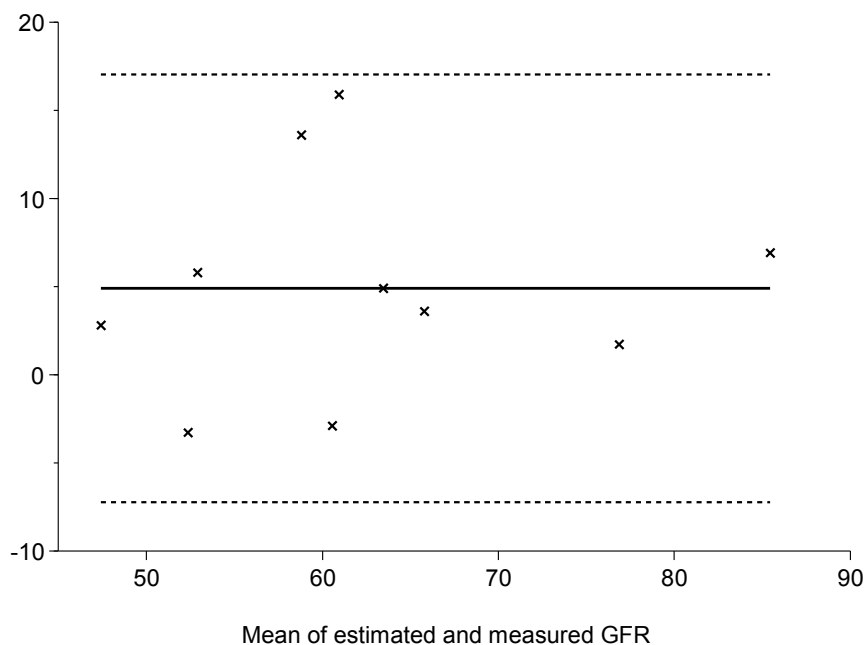


Figure 17. Bland-Altman plot for difference in mean eGFR (CKiD III) minus mGFR (solid line) and 95% limits of agreement (dashed lines; difference $\pm 1.96 \times \text{SD}$) in ten pediatric CLKT patients at last follow-up (not included in IV).

Table 18. Renal function for CLKT and KT patients one and five years after transplantation

	Year 1	Year 5
	Mean (SD)	
Measured GFR		
Pediatric CLKT patients	63 (17)	61 (19)
Pediatric KT patients	55 (19)	50 (20)
Difference (95% CI)	8 (-7 to 23)	11 (-4 to 26)
Estimated GFR		
Adult CLKT patients	75 (26)	62 (21)
Adult KT patients	67 (24)	61 (24)
Difference (95% CI)	8 (-5 to 20)	1 (-10 to 12)

Number of patients 9 and 10 and 21 and 23 at one and five years for pediatric CLKT and KT patients, respectively. Number of patients 23 and 21 and 69 and 63 at one and five years for adult CLKT and KT patients, respectively.

CLKT; combined liver-kidney transplantation, GFR; glomerular filtration rate, KT; kidney transplantation

6. Discussion

In this thesis, several long-term findings were made in the context of aims. Namely, the prevalence of hepatic artery thrombosis after pediatric liver transplantation was higher than what has previously been observed in the literature. In contrast, the prevalence of donor-specific antibodies after pediatric LT was comparable to what has been observed in the literature. Long-term renal function after pediatric liver transplantation remained stable for five years but started to deteriorate thereafter, which is in line with some studies. In addition, long-term renal function remained stable in pediatric but was impaired in adult CLKT patients, an important observation due to the limited literature.

Patient survival for pediatric LT and CLKT patients reported is inferior or comparable to what has been observed in large registry-based or single-center studies (4, 113). For instance, a survival probability of 69% at ten years after LT was observed in this cohort compared to 78% observed in a large single-center study with over 1,000 LTs (4). In contrast, 81% of pediatric CLKT patients were alive at ten years after transplantation, a survival estimate that is slightly better than the 79% observed in a large registry-based study (113). Adult CLKT patients seem to have superior survival compared to at least one previously published large registry-based study (115).

6.1 Late HAT (I)

Fifteen patients were observed to have hepatic artery thrombosis after LT, defined as late HAT because of the time interval since transplantation. However, the exact time when thromboses in these 15 patients were formed remains inconclusive due to the cross-sectional nature of the study (I).

The true prevalence of late HAT can be lower or higher compared to what was observed, since 32 potential patients were not studied. If one assumes that all these 32 patients would have late HAT in addition to the 34 studied patients, then prevalence would be as high as 71%, but then again, it would be as low as 23% if one assumes that none of these 32 patients who were not studied would have HAT.

Altogether, the observed late HAT prevalence of 44% is higher than in the previous published literature, even if the focus is only on late HAT diagnosed with ultrasonography. Many late HAT studies, especially with pediatric patients, are relatively old or the incidence of late HAT has not been reported separately for pediatric patients. A study with pediatric patients transplanted between 1989 and 1994 showed a late HAT prevalence of 3% (2/73), although this prevalence was not directly reported albeit calculable (60). According to an editorial related to our study, no late HAT cases were observed at the time in 40 pediatric LT patients operated in 2008 (174).

Donor-to-recipient (DR) weight and age ratio was higher in our patients with late HAT, which is at odds with a pediatric study on early HAT which showed higher donor-to-recipient weight and age ratio for patients without HAT, although the differences in medians between groups were 0.7 and 1.6 for weight and age ratio, respectively (62). Several factors associated with HAT have been proposed in a way that some risk factors are concordant and some discordant across studies (46). However, there is no standardized way for conducting statistical analyses considering risk factors, especially using regression methods with small samples or adjusting P-values for multiplicity, as was done in study I.

There was around a three-year difference in the duration of acetylsalicylic acid treatment in patients with and without late HAT (I). However, when focusing on those fifteen patients in whom the duration of ASA treatment was based on clinical decision, instead of the date of MRI, the difference in treatment duration between late HAT and no late HAT patients was less pronounced (68 versus 92 days). Four patients did not receive ASA at all (one in late HAT and three in no late HAT groups).

As pointed out earlier in the literature review, pooling two studies (70, 71) yielded inconclusive results considering an association between aspirin usage and the incidence of late HAT. A pediatric study showed that 73% of patients with early HAT compared to 87% of patients without early HAT were on antiplatelet therapy (175). The efficacy of antiplatelet therapy for reducing the risk of HAT in pediatric patients remains to be proven although it is widely used (73).

Poor patient outcomes in late HAT patients were not evident by cross-sectionally analyzed biochemical and histological variables although some discrepancy on timing with MRI was evident (I). Most patients had collaterals, which might provide enough blood flow to the liver graft and protect against more drastic features of late HAT, the point addressed in guidelines (97). These collaterals might also explain the false negative findings with ultrasonography, which led to diminished sensitivity. However, even asymptomatic adult patients with late HAT can develop severe symptoms, which eventually lead to graft loss and need for re-transplantation (51). Naturally, the course of late HAT can be different in pediatric than in adult patients, although late HAT did play some part in one patient's graft loss also in our study.

The long-term outcomes of pediatric late HAT patients remain open, and the need for closer surveillance of these patients should be balanced against benefits and harms.

6.2 DSA (II)

Approximately half of the patients had donor-specific antibodies, mainly against class II HLA antigens. This prevalence estimate is in line with similar-sized studies and with the use of a mean fluorescence intensity of 1,000 for positivity (8). Couchonnal and coworkers showed in 100 pediatric patients DSA prevalence of 24% with the use of MFI 500 for positivity, albeit the prevalence rose with time (7). MFI values for class II DSAs were, on average, over 10,000, which is commonly observed for DSAs within this class (7, 159).

Transplantation and CNI type at the time of DSA detection showed the strongest associations for DSA-positivity in univariate analyses (II). These two factors were further accounted for simultaneously, which led to lower odds ratios but wider confidence intervals. Additionally, in further analysis, follow-up time was associated with higher odds for having DSAs, but less so when age at the time of transplantation was also adjusted.

Couchonnal and coworkers similarly showed in a univariate analysis protective association of CLKT and harmful association of cyclosporine for DSAs with MFI over 10,000 (7). However, these factors were not included in the final regression model based on a P-value threshold of 0.10. On the contrary, follow-up time since LT was associated with increased

odds for DSAs even after adjusting for six other variables (7). Nonetheless, there were only 14 DSA-positive patients with MFI over 10,000 leading to so-called events per variable of two with a total of seven adjusted variables (7). In other words, there were only two DSA-positive patients with MFI over 10,000 for every adjusted variable; therefore it is problematic to use standard logistic regression methods with such a low number of events per variable (160).

In our study, portal inflammation was more distinct in DSA-positive than in DSA-negative patients. This is an observation that has to some extent been highlighted by others as well (7, 159). Couchonnal and coworkers showed that all DSA-positive patients with biopsies had inflammation compared to 89% of DSA-negative patients (7). In addition, some form of inflammation was observed in 63% of DSA-positive and 54% of DSA-negative patients (159). This underscores that portal inflammation is also prevalent in DSA-negative patients; nonetheless, only one DSA-negative patient had portal inflammation in study II. Recently, Feng and coauthors demonstrated with pediatric LT patients followed for, on average, nine years that class II DSAs were associated with higher odds of belonging to cluster defined by portal inflammation with interface activity (153).

In contrast to inflammation, fibrosis has been more often associated with DSAs, especially centrilobular fibrosis (7, 159). Some form of fibrosis has been shown to be present in all DSA-positive compared to 69% of DSA-negative patients (159). A more recent study showed centrilobular fibrosis in 65% and 29% of DSA-positive and DSA-negative patients, respectively (7). In our study, fibrosis was also more evident in DSA-positive patients although mostly deemed to be mild, and the confidence interval also included the possibility for lower prevalence (I). However, all three studies point in the same direction. The more severe forms of fibrosis can be due to longer-follow-up (7) although the correlation between follow-up time and severity of fibrosis was weak (159). Nonetheless, repeatedly assessed protocol liver biopsies after pediatric LT underpin the progression of fibrosis over time (176).

The long-term significance of DSAs after pediatric LT is still uncertain. The study by Couchonnal and coworkers showed that graft survival was comparable in DSA-positive and -

negative patients although the survival curves crossed more than a decade after LT (7). In addition, some DSAs could be more harmful than others. Indeed, complement-activating DSAs have been associated with graft loss in solid organ transplantations (177). However, this systematic review by Bouquegneau and colleagues included only four LT studies (177). One of these was the aforementioned pediatric study, which showed comparable graft survival for C3d-binding and non-C3d-binding DSAs although the survival curves crossed more than a decade after LT (7).

Although associations between DSAs and liver histology were found (II), their clinical meaning remains indistinct. There are many open questions considering the role of DSAs in a pediatric LT setting, including screening for DSAs and understanding the significance of MFI (178, 179). Several possibilities and opportunities have been underscored considering DSAs in LT setting (180).

6.3 Renal function (III, IV)

The observation of stable renal function up to five years after pediatric LT and declining GFR thereafter extends the results observed at our institution in 1994 (86). Laine and colleagues showed that mGFR was around 120 three years after LT with six patients. In contrast, 45 patients were available (III) at three years with lower mean GFR (III) than observed by Laine et al (86). However, all measured GFRs taken before 2006 were corrected with the method of Bröchner-Mortensen, which leads to lower GFRs, especially with higher values (16), in such way that mGFR 120 would be 99 after the correction.

Long-term studies with the use of different measurement methods have shown declining or stable renal function up to ten years after pediatric LT (6, 84, 85). On average, GFRs were lower during follow-up (III) compared to studies shown in Table 4 [chapter 2.2.6.3], especially the one (85) which also used the same GFR measurement method that was utilized in Study III. In contrast, another pediatric study showed a comparable mGFR as observed in our study at ten years after transplantation also applying the same measurement

method (95). Biliary atresia patients had better renal function at one year after LT (III) compared to other diagnoses; the effect of primary diagnosis has also been observed by others in pediatric LT patients (6, 83, 88).

Renal biopsies revealed that there were no CNI-induced histologic changes in four-fifths of the biopsy specimens. In addition, when histologic changes were present, they were mostly mild. This finding is in line with those in adult LT patients with renal biopsies taken with a mean 5 years after transplantation (181). In our study, the most commonly observed histologic abnormality was isometric vacuolization, which has also been observed to be more prevalent within the first six months than later in pediatric kidney transplant patients, indicating its temporary course (182).

Contrary to what was observed with BSA-corrected GFR, absolute GFR improved in patients less than five years of age at the time of LT (III). However, in these patients BSA-corrected GFRs started to decline after one year, which was not observed in older patients (III). Renal growth might be insufficient in these younger patients after LT. Nonetheless, Herlenius and colleagues also reported improvement of absolute GFR, but with stable BSA-corrected mGFR (85). According to Dubourg and colleagues, pediatric patients who receive kidney grafts from pediatric donors are able to improve absolute mGFR, resulting in a relatively stable BSA-corrected mGFR, but this pattern was not evident with kidney grafts from adult donors (183).

The ease of using GFR estimation equations is reflected with biased estimation of measured GFR, which was observed in both studies included in this thesis that focused on renal function (III, IV). Overestimation was, on average, considerable with older estimation equations, like the Schwartz equation (167) used in (III). Other pediatric LT studies have also reported overestimation of mGFR with the Schwartz equation (88, 89, 95) although some studies show regular underestimation in children transplanted at less than two years of age (87). The overestimation was less pronounced with the Counahan-Barratt equation (III), which resembles the bedside Schwartz equation used in study IV. The constant in these equations is very similar, although the GFR measurement method used for equation devel-

opment was different. Bedside Schwartz equation also showed, on average, overestimation of measured GFR after CLKT (IV). Some centers have modified the bedside Schwartz equation with introduction of two constants depending on gender and age (184).

In studies III and IV, a cystatin C and urea-based equation was also used. This resulted in less bias than creatinine-based equations although only more thoroughly compared to mGFR at last follow-up in ten pediatric CLKT patients. Nonetheless, this equation is rather complex, at least for routine clinical use, albeit performing better than bedside Schwartz for longitudinal renal function assessment in pediatric KT patients (96). Naturally, any GFR measurement method can be biased compared to the reference method of renal inulin clearance (15). However, plasma clearance of ^{51}Cr -EDTA fulfills the criteria of a sufficiently accurate method for GFR measurement based on moderately strong evidence (15).

In contrast to pediatric LT patients (III), renal function remained stable in pediatric CLKT patients up to ten years after transplantation (IV). This observation is at odds with the longitudinal follow-up studies shown in Table 5 [chapter 2.3.2.2]. Estimated GFRs in two of these studies (109, 110) declined from 13 to 18 mL/min/1.73 m² between one to ten years after CLKT, respectively. On the contrary, eGFR increased within the same time-period in (IV). Neither of these two studies used measurement of GFR, and the patients were, on average, older than in (IV) with mean or median ages 7 to 12 at the time of CLKT (109, 110). One study showed stable eGFR in their primary hyperoxaluria patients between one and ten years after CLKT (109) while the other showed impairment of renal function in their primary hyperoxaluria patients although eGFR stabilized after three years (110). Only two primary hyperoxaluria patients were in (IV), and renal function improved in these two patients with their native kidneys (IV).

Average renal function was better in pediatric CLKT patients compared to matched pediatric KT patients (IV). Nonetheless, GFR remained stable in these matched patients as well, with a similar pattern as observed in CLKT patients. Ranawaka and co-authors showed declining eGFR in both CLKT and KT patients albeit GFR was also better in CLKT patients (109). Donors for pediatric KT patients were older compared to donors for pediatric CLKT patients,

which might reflect on the higher GFR levels observed in CLKT patients. However, it is reasonable to assume that older kidney grafts would also mean declining GFR instead of stable GFR at least up to five years after KT (185). Still, the inclusion of donor age did not improve model performance for GFR prediction in a small sample of pediatric KT patients (186).

In adult CLKT patients, eGFR declined during follow-up. A large registry-based study observed that evolution of GFR depended on primary liver disease (115). However, eGFRs were around 60 during follow-up when these disease groups were pooled [Table 6 in chapter 2.3.2.2], which is comparable to that observed in our study IV. Other studies have also shown stable eGFR around 50 to 60 up to five years after CLKT (123, 133). Nonetheless, eGFR declined initially from one to three years after CLKT (IV) for unknown reasons. Relatively high cyclosporine trough levels at one year might explain this drop in eGFR, although this is merely speculative. As observed with longer follow-up up to ten years after CLKT, renal function further deteriorates (IV). To my knowledge, there are no studies with follow-up up to ten years or beyond in adult CLKT patients.

Similarly, a declining GFR pattern was also observed with matched adult KT patients. Taner and coworkers also showed that GFR remained stable in CLKT patients but deteriorated in DSA-positive KT patients (123) although GFRs were, on average, lower compared to what was observed (IV). CLKT has been reported to be associated with lower chances of combined end-point of graft loss or GFR decline over 50% (123). Unfortunately, GFR was not analyzed continuously. In contrast, transplantation type had a clinically negligible effect on renal function when GFR was analyzed continuously in adults with linear mixed models in (IV).

6.4 Limitations and strengths

Studies I and II were cross-sectional by nature, thus providing only a snapshot of the current radiological, immunological and histological status of the patients, which limits any conclusions drawn before and after the evaluated time-point. In addition, there was some divergence in time between MRI or DSAs and liver biopsies as well as laboratory tests. In study I, almost half of potential patients were not included, which can create sampling bias albeit

MRIs were taken without clinical indications from all except one patient. In study IV, control patients were not selected randomly, which might introduce sampling bias. All studies were based on a relatively small sample of patients, which nonetheless led to the use of statistical methods more suitable for small sample sizes.

The strengths of these studies include detailed analyses of different factors (I, II), measurement of GFR (III, IV), use of renal biopsy specimens (III) and control patients (IV), and a unified treatment protocol for caring for all patients (I, II, III and IV).

From a statistical perspective, P-value threshold of 0.05 for statistical significance was used in study III. However, conclusions from scientific results should be not based on dichotomized P-values, as has been pointed out by the American Statistical Association (187) as well as notified in transplantation literature (188). Indeed, if the assumptions of the underlying statistical model are wrong, then P-values are irrelevant even if they pass an arbitrary threshold for statistical "significance".

6.5 Future prospects

The transplantation era in three of these studies (I, II, III) ended in 2007, which would naturally allow to extending follow-up with more observations, especially considering GFR measurements, and more full comparison of different GFR estimations equations. In addition, a follow-up study of late hepatic artery thrombosis patients would allow observing whether these patients have remained asymptomatic or not after several years have passed. Nonetheless, single-center studies might not be able to capture all aspects of late HAT reliably if one assumes that true prevalence is much lower than observed (I). As pointed out in an editorial published alongside our study I (174), this observed prevalence of late HAT should be replicated with other studies, which I wholeheartedly support and wait for. It would also be interesting to study whether DSAs have persisted, to what extent, and whether this is associated with graft survival in these patients. Generally, more RCTs and systematic reviews should be conducted in the field of pediatric liver transplantation since only approximately 1% of the literature cited in pediatric LT guidelines are RCTs and systematic reviews (189).

The definition of meta-research is study of research itself, which can include various aspects, such as how results are reported, how guidelines are made or what statistical methods are used (190). This thesis also generated the idea that the field of transplantation provides a unique opportunity to conduct research on research.

7. Conclusions

Based on this thesis, the following conclusions can be drawn

- Late hepatic artery thrombosis is common after pediatric liver transplantation. No severe aspects were found in terms of histological or biochemical variables in relation to late hepatic artery thrombosis.
- Donor-specific HLA antibodies are common after pediatric liver transplantation and were associated with portal inflammation.
- Longitudinal renal function remains stable after pediatric liver transplantation but declines after five years.
- Longitudinal renal function is steady after pediatric combined-liver kidney transplantation but declines in adult patients.

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